ASSOCIATIVE DIASCHISIS AND SKILLED REHABILITATION-INDUCED BEHAVIORAL RECOVERY FOLLOWING FOCAL ISCHEMIC INFARCT.

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DEDICATION

I would like to dedicate this thesis to my husband Jamie who understands that science does not stop for holidays. Also, to my parents John and Linda, who showed me that being a workaholic pays off in the end. To my brother, James who also likes to rant about academia.

ABSTRACT

The time course of peri-infarct diaschisis following a focal ischemic infarct and the effects of delayed rehabilitation on behavioral and functional recovery were examined. Intracortical microstimulation (ICMS) was used to derive topographical maps of forelimb representations within the rat motor cortex and ischemia was induced via bipolar coagulation of surface vasculature. At one hour there was a dramatic expansion of representations in control but not ischemic animals. A significant loss of forelimb representations within peri-infarct cortex was observed in ischemic animals at twenty-four hours after insult. Delayed rehabilitation alleviated diaschisis in peri-infarct cortex. A lack of rehabilitation prevented functional and behavioral recovery. The rapid development of peri-infarct dysfunction indicates the need for immediate administration of therapeutic interventions following an ischemic event. These results indicate that the timing of rehabilitation does not effect functional and behavioral recovery but does support the need for rehabilitative interventions to facilitate these types of recovery.

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CHAPTER 1:

GENERAL INTRODUCTION

The most common cause of brain damage is stroke. In Canada, there are between 40,000 and 50,000 strokes each year and there are currently 300,000 Canadians living with the effects of stroke. Stroke costs the Canadian economy $2.7 billion per year and the average costs for acute care is $27,500 per stroke. There are three basic approaches to stroke treatment; prevention, neuroprotection, and rehabilitation. Preventative therapies have tried to eliminate the occurrence of all strokes which is not an effective strategy because it is impossible to control for many of the factors such as age and genetics that contribute to stroke incidence. At this time, there are very few neuroprotective agents which have proven effective a few hours post-stroke (Madden, 2002). Rehabilitation seems to be the only effective strategy to assist in the recovery from motor impairments following stroke. The majority of stroke victims experience some type of movement disability including facial and limb hemiparesis. Although twenty percent of stroke survivors are permanently disabled, many stroke patients are able to regain functional independence through rehabilitation. Despite the widespread use of rehabilitation, the neural mechanisms underlying motor impairments are poorly understood.

Stroke or ischemia, is the deprivation of blood to tissue that results in cell death. The area of cell death is referred to as the infarct and surrounding areas are considered peri-infarct areas. In the early 1900s, von Monakow introduced the term diaschisis to explain the subsequent functional depression of non-ischemic brain areas connected to the locus of damage (von Monakow, 1914). Since the early 1900s, diaschisis evolved to

include changes in cerebral blood flow, excitability of neural tissue, and metabolism leading to the presence of behavioral impairments following stroke. Current research has led to the belief that diaschisis can be overcome with the administration of rehabilitative interventions and that the alleviation of functional depression may facilitate behavioral recovery. It is this theory that the present thesis is based.

Changes in neural function associated with diaschisis and behavioral recovery following rehabilitation are difficult to directly study in a laboratory. To investigate this relationship, a model is needed that provides a method of viewing specific changes in neural function related specifically to behavioral performance following ischemic injury. Kleim and others have developed a rat model to provide the method by which we can investigate diaschisis and rehabilitation-induced recovery following stroke (Goertzen et al., 2001). In this model we are able to define the functional organization of the motor cortex, produce an ischemic infarct that causes motor impairments, administer rehabilitation to promote recovery, and re-examine functional organization.

This thesis introduction will describe the neural progression of changes associated with ischemic injury. Behavioral deficits associated with ischemia will also be reviewed. The notion that peri-infarct diaschisis accounts for the behavioral deficits and alleviation of diaschisis is related to functional recovery will be discussed. Further, the model used to test the theory will be described. Finally, an outline of the questions addressed in this thesis will be provided.

ISCHEMIC INJURY

In order to understand the neural mechanisms of behavioral impairments it is important to keep in mind the cellular consequences following stroke. Behavioral neuroscientists assume that behavioral performance depends on the functional properties of the nervous system. When damage to the nervous system occurs there are changes to the functional properties of the area of study, for example, the motor cortex and behavioral deficits emerge. If the areas surrounding a specific area of the motor cortex are being investigated, it is also important to remember what processes are present following damage. In the two experiments included in this thesis, the methodology used to measure neural performance depends directly on the properties of the motor cortex. For the purpose of this thesis, a review of the events following ischemic injury are reviewed to illustrate what processes are present following stroke possibly facilitating rehabilitation-induced recovery.

Ischemia describes an event in which biological tissue is deprived of blood flow. The consequence of ischemia is a lack of oxygen and other necessary metabolites resulting in tissue loss. Ischemic strokes can be either thrombotic or embolic. A thrombotic stroke results from a blood clot or thrombus that forms in an artery going to the brain. Embolic ischemia occurs when a brain artery is clogged by a clot formed elsewhere in the body (embolus) and carried via the blood stream to the brain. According to the Heart and Stroke Foundation of Canada, twenty percent of strokes are hemorrhagic. This type of stroke is caused by uncontrolled bleeding in the brain. Subarachnoid hemorrhages occur when bleeding is present on the surface of the brain and intracerebral hemorrhages result from ruptures of arteries deep within the brain.

Hemorrhages are most likely caused by structural problems of blood vessels in the brain. Stroke-like damage can also be caused by cardiac arrest. Ischemic damage can either be a global or focal event. A global ischemic event can arise from cardiac arrest resulting in the cessation of blood flow to the entire brain. Focal ischemia arises from the occlusion of a blood vessel(s) in the brain. The cessation of blood flow may be a transient or permanent phenomenon. In a transient focal ischemic model neuronal damage presents rapidly where the boundaries of the lesion are visible at twenty-four hours (Chen et al., 1993). However, in permanent ischemic models the volume of lesion is maximal six to twelve hours post lesion (Garcia et al., 1993).

Focal ischemic events result in the disruption of many processes within the brain. Neuronal degeneration following focal ischemia has been proposed to take up to two or three days (Du et al., 1996). Focal ischemic injury influences a core infarct area of tissue that is deprived of blood flow in which primary cell death occurs. The infarct area experiences dramatic permanent decreases in cerebral blood flow and oxygen-deprived depolarizations in the minutes post stroke (Dirnagl et al., 1999). The core area is unable to perform most normal cellular functions. Immediately surrounding the core is the peri-lesional or penumbral region, which experiences decreased blood flow. Regional blood flow levels in the peri-lesional area are below that required to sustain normal neuronal activity but above that required to maintain energy metabolism (Witte and Stoll, 1997; Dirnagl et al., 1999). Other terminology used to describe the penumbra include "peri-infarct" (Neumann-Haefelin and Witte, 2000) or the "surround" (Buchkremer-Ratzmann et al., 1998; Hagemann et al., 1998). Neurochemical changes in the penumbra include increases in oxygen extraction, acidosis due to an imbalance in acid-base homeostasis,

high glucose utilization, and decreased levels of adenosine 5'-triphosphate (ATP) (Obrenovich, 1995). These penumbral conditions exist in a small transient zone outside the infarct core and there is a tendency for the penumbra to evolve towards cell death associated with the core over time (Dirnagl et al., 1999). Although this area is dysfunctional, it retains the potential for functional recovery (Symon et al., 1977).

Necrosis and apoptosis are the two types of cell death that occur within the core of the infarct and the peri-lesional region, respectively. Necrosis is a passive event that occurs due to ATP depletion, leading to a failure of ionic pumps, secondary neuronal swelling and lysis resulting in leakage of intracellular components into surrounding tissue followed by inflammatory response activation and edema (Lewen et al., 2000). Apoptosis results from a genetically regulated program which induces cells to die with minimal release of genetic material and other inflammatory intracellular constituents (Johnson & Deckwerth, 1993). These neurons also exhibit chromatin condensation and aggregation to the nuclear margin, cell and organelle shrinkage, and fragmentation of the nucleus and cytoplasm into membrane bound vesicles (Kerr et al., 1972). The core and the peri-lesional area undergo different types of cell death because of the level of oxidative stress experienced. A high level of oxidative stress associated with minimal levels of blood flow results in necrosis but low levels result in apoptosis (Gardner et al., 1997). The infarct core experiences a high level of oxidative stress and cell death has been hypothesized to be due to necrosis (Heiss & Rosner, 1983). Peri-lesional cell death results from delayed apoptosis (Dirnagl et al., 1999) due to lower levels of oxidative stress.

STAGES OF TRAUMA

Following an ischemic event, the core and peri-lesional tissue endure a progression of stages of trauma. The stages of trauma following stroke can be placed into categories that are defined by the time at which specific neurochemical or physiological changes occur. Most timeline divisions are based on changes in electrical activity, cerebral blood flow, and cerebral metabolism, however, specific behavioral impairments can be associated with the time from which the ischemic event occurred. The study of physiological changes is important to provide an explanation for the neural mechanisms associated with behavioral impairments and therefore provide an explanation for behavioral recovery following rehabilitation. Although as many as five stages of trauma have been proposed (Andrews, 1991), for the purpose of this thesis three phases of ischemia will be described because of the difficulty of separating processes by time periods in which they occur. The first phase is a combination of the hyperacute and acute phases of ischemia, which include the time of an ischemic event to twenty-four hours following cerebral injury. The second phase, the subacute phase, spans one to ten days. Finally, the third phase or the chronic phase, is the combination of intermediate and chronic phases including anything after ten days. The divisions of the timeline do not represent clear-cut lines but are divided according to definitive events in each phase. There is overlap of different processes within the phases of stroke.

Acute Phase (0-24 hours)

In the acute phase, there are a variety of processes that occur immediately after or within twenty-four hours following an ischemic event (Figure 1.1). The acute phase of stroke initially triggers various processes, mechanisms, and events that determine the

formation of a lesion (Witte et al., 1997). The most important of these processes include excitotoxicity, calcium signaling, and peri-infarct depolarizations. Physiological changes are associated with a variety of initial behavioral impairments. The Heart and Stroke Foundation of Canada lists warning signs of stroke present at onset including weakness in the extremities, temporary loss of speech or ability to understand speech, visual impairments, and loss of balance. Within five hours of an ischemic event behavioral impairments may progress to bilateral sensory or motor deficits, disorders of eye movement, or crossed syndromes (Argentino et al., 1996). Behavioral impairments as listed above usually persist throughout the acute stage without much change.

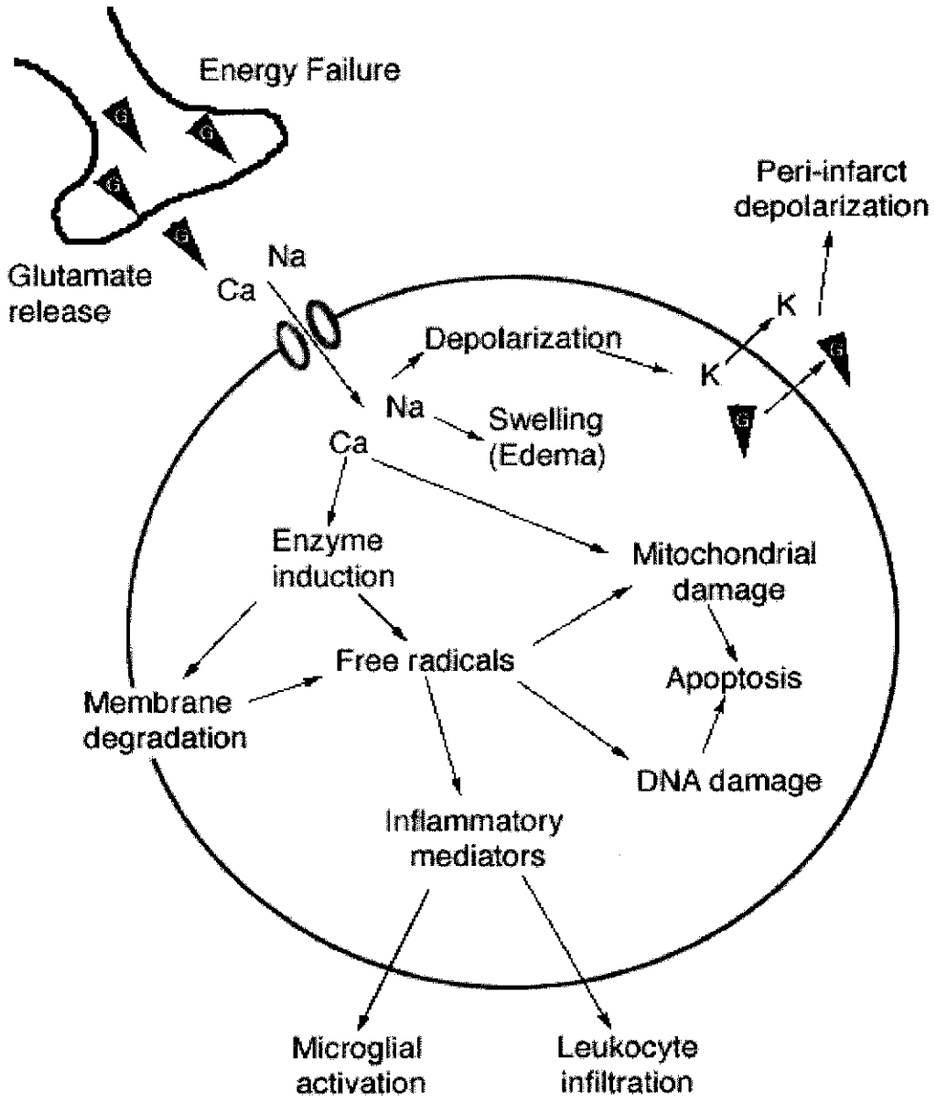

Figure 1.1 - Overview of acute phase mechanisms following ischemia. Energy failure results in glutamate (black triangle) release, increasing intracellular calcium (Ca) and sodium (Na). Potassium (K) is released into the extracellular space. Changes in ion concentrations induce peri-infarct depolarizations and edema. Calcium activates enzyme systems, free radicals are generated, and apoptosis occurs. Free radicals activate an inflammatory response. (Adapted from Dirnagl et al., 1999).

i. Excitotoxicity

During cerebral ischemia there is an abrupt decrease in blood flow to a brain area restricting oxygen and glucose delivery. Almost immediately there is a decrease in high-

energy metabolites such as ATP and phosphocreatine (Small et al., 1999). This results in an energy crisis in which ATP hydrolysis exceeds ATP production and a significant amount of acidity is produced (Saplosky, 1992b). Acid production is then further enhanced by the anaerobic nature of metabolism during oxygen deprivation. At this point, lactate begins to accumulate (Small et al., 1999), the pH declines, and protein synthesis is altered (Sapolsky, 1992a). Neuron death is not linked to a lack of energy generation but possibly to an over production of the anaerobic energy causing an increase in the production of cellular toxins.

When the cells are depleted of energy, membrane potentials are lost and neurons depolarize because the energetics required to maintain ionic gradients are impaired (Dirnagl et al., 1999). With the loss of sodium (Na^+) gradients, a depolarization of the cell triggers calcium (Ca^{2+}), Na^+, chloride (Cl^-), and water influx (Witte et al., 1997) via voltage and ligand-gated channels. This further depolarizes the membrane and stimulates the release of massive amounts of the excitatory neurotransmitter glutamate into the extracellular space (Small et al., 1999). Further, potassium (K^+) is expelled from the neurons (Witte et al., 1997) causing an excessive influx of Cl^- and Na^+, which then pulls in large amounts of water (Sapolsky, 1992a). The increase in intracellular volume causes the cells to swell resulting in edema and cellular burst (Dirnagl et al., 1999). After approximately one hour all neurons within the core have been irreversibly damaged (Witte et al., 1997). Histological sections show that at five hours post stroke there are structural alterations such as neuronal degeneration likely caused by edema (Scheine et al., 1996).

Excitotoxicity of neurons is dependent upon the excessive release of glutamate from presynaptic nerve terminals into extracellular space which overstimulates glutamate receptors, especially *N*-methyl-D aspartate (NMDA) receptors (Zipfel et al., 2000). In turn, this overstimulation leads to an excessive influx of Ca^{2+} and Na^{+} through glutamate receptor-gated ion channels followed by passive movements of Cl^{-} and water. Energy-dependent mechanisms for glutamate reuptake are impaired causing prolonged activation of glutamate receptors and resulting in further Ca^{2+} influx through activation of Ca^{2+} channels (Dirnagl et al., 1999; Small et al., 1999). The increase of intracellular Ca^{2+} from normal resting levels has been proposed to activate other processes and mechanisms that may eventually lead to necrosis or apoptosis (Small et al., 1999).

ii. Calcium Signaling

With energy depletion resulting from decreased blood flow, intracellular Ca^{2+} dramatically increases. It is no longer sequestered within the mitochondria of neurons because of a loss of ATP production (Small et al., 1999). Therefore, excess intracellular Ca^{2+} is the result of a failure to remove it from the cytosolic compartment (Sapolsky, 1992a). This resulting increase in Ca^{2+} and cellular volume induces lethal metabolic derangements, internal organelle swelling, and plasma membrane failure (Choi, 1995) consistent with necrosis.

Increased intracellular Ca^{2+} can also stimulate various second messenger systems that contribute to cell death. As a result of the disruption of Ca^{2+} homeostasis, there is activation of a series of Ca^{2+} dependent processes. The levels of Ca^{2+} during the acute phase of stroke initiate the activation of certain enzymes, which carry out destructive actions during the subacute phase. Such proteolytic enzymes include kinases and

proteases. Also activated are immediate early genes (IEGs), cytokines, and cell adhesion molecules (Small et al., 1999). The activation of second messenger pathways results in the delayed production of free radical species which further damage cells and lead to apoptosis in the subacute phase.

iii. Peri-infarct or Spreading Depolarizations

Neurons surrounding the core of energy and oxygen depletion may experience peri-infract or spreading depolarizations (SDs) as a result of the disruption of calcium homeostasis. SDs are transient suppressions of electrical activity with membrane depolarizations propagating across the cortex in regions not directly affected by ischemic insult (Lauritzen, 1994). As mentioned above with the loss of ionic gradients, K^+ and glutamate are expelled from neurons. Normally, K^+ channels contribute to the resting potential of neuronal membranes and activation of these channels helps to maintain the hyperpolarized potential (Small et al., 1999). In the areas surrounding the infarct core, where perfusion of blood is preserved, cells are able to repolarize. Due to the excessive amounts of extracellular K^+ and glutamate, however, continuous depolarizations are likely to occur (Dirnagl et al., 1999). Repetitive depolarizations may occur for six to eight hours post-ischemic insult and are thought to increase in number as the size of the lesion increases (Dirnagl et al., 1999). It has not been determined whether depolarizations directly contribute to cell death or just cell dysfunction.

Subacute Phase (1-10 Days)

In the days following an ischemic insult, both the brain areas directly affected by ischemia and the adjacent areas experience dramatic changes initiated by processes in the acute phase of ischemic injury. In the subacute phase, the processes of enzymatic

damage, inflammation, and excitability shifts all contribute to apoptosis by changing normal cellular function (Figure 1.2). Also characteristic of the subacute phase are specific behavioral aberrations which can be evaluated to assess the progression of ischemic damage. For example, the Canadian Neurological Scale is used to evaluate levels of consciousness, speech, and facial and limb strength in addition to many other skills which persist in some form through the different phases following stroke (Cote et al., 1986). Half of the time patients were well oriented and had normal speech. However, some patients exhibited expressive deficits, had receptive disorders, and a majority had at least one motor deficit in the face, arms, or legs (Cote et al., 1986). During this phase, there is definite formation of a lesion and a slow progression of the infarct to which behavioral deficits may be attributed.

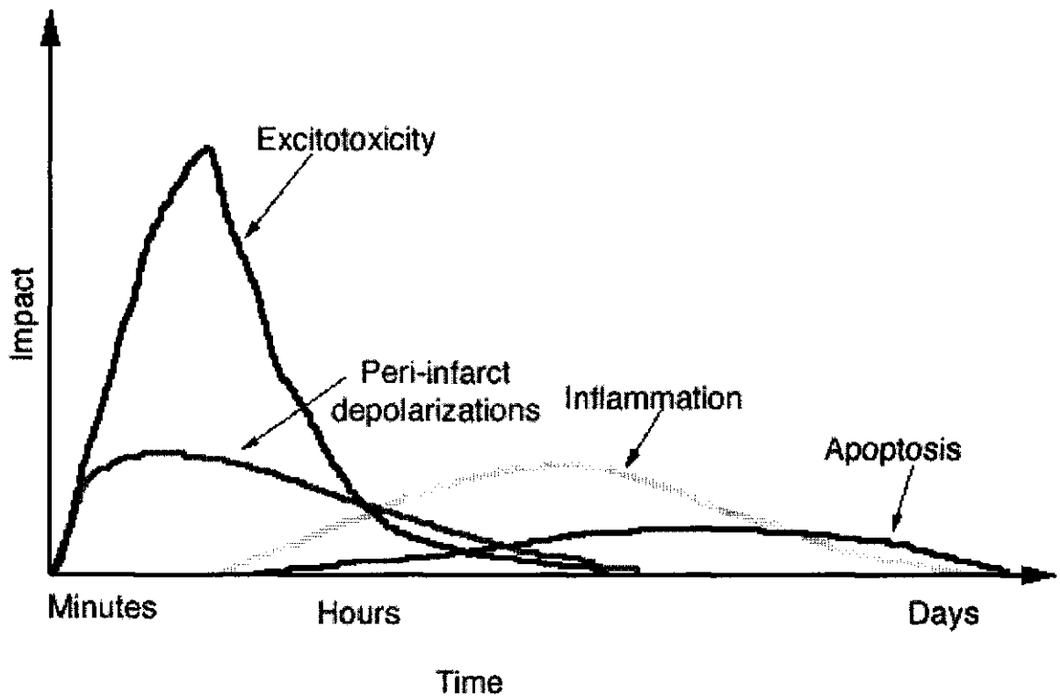

Figure 1.2 - Cascade of events in the subacute phase of stroke. Initially following blood blockage excitotoxicity damages neurons and glia. Excitotoxicity also induces other events that contribute to the death of the tissue. These events include peri-infarct

depolarizations, inflammation, and apoptotic cell death. The x-axis represents the evolution of events over time and the y-axis portrays the degree of impact the event has at that time following ischemia. (Adapted from Dirnagl et al., 1999).

i. Enzymatic Damage

Hours to days post-stroke, the disruption of Ca^{2+} homeostasis leads to the activation of a series of Ca^{2+} dependent processes that most likely culminate in secondary injury and cell death. Enzymatic damage is the result of the second messenger systems activated by calcium signaling in the acute phase. These systems activate various degradative enzymes, participate in free radical production, and induce immediate early genes. Among the enzymes activated are the kinases, which are a family of signaling enzymes that affect cell proliferation, differentiation, secretion, and death (Small et al., 1999). During ischemia, there is an increase in kinase activity by translocation to the plasma membrane or nucleus where target proteins are phosphorylated (Chakravarthy et al., 1998). There is an activation phase followed by a long lasting loss of activity. The second type of enzymes activated are the proteases. Proteases are proteolytic enzymes that degrade cytoskeletal proteins like actin and spectrin and extracellular matrix proteins like laminin (Dirnagl et al., 1999). This uncontrolled proteolysis leads to structural failure of the cell.

Another result of enzymatic activation is the resulting generation of free radical species. Free radicals are atoms or groups of atoms with unpaired electrons that can form indiscriminate chemical bonds with a variety of molecules, thus altering their function (Sapolsky, 1992a). For example, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated by Ca^{2+} activation (Lewen et al., 2000). Normally, oxygen radicals generated during oxidative phosphorylation by the electron transport chain are

contained by oxygen radical scavengers (Sapolsky, 1992a). ROS formation may be due to Ca^{2+}-dependent alterations of the inner mitochondrial membrane resulting in disorganization of the electron transport chain (Kowaltowski et al., 1995). When the membrane becomes more permeable, there is swelling, cessation of ATP production and an oxygen free-radical burst (Dirnagl et al., 1999). RNS specifically decreases neuronal energy production by inhibiting glycolytic and mitochondrial enzymes increasing DNA damage and conversion of other toxic free radicals (Bredt and Snyder, 1994). Twenty-four hours post-ischemia free radicals necessary for acid metabolism are increased in the entire injured hemisphere decreasing slightly over the next two to five days (Bidmon et al., 2000). The energy depletion associated with an ischemic insult compromise the capacity of the cell to correct this oxidative and enzymatic damage.

ii. Inflammatory Response

In the acute phase of ischemia the inflammatory response is initiated by the dramatic increase of intracellular Ca^{2+}, excessive release of excitatory acids, activation of cell adhesion molecules, and cellular infiltration (Witte et al., 1997). The inflammatory response is dominated by the recruitment of neutrophils, whose presence peaks at day one post-stroke (Schnell et al., 1999). Neutrophils are the first cells to arrive at the site of the lesion and their function is to remove microbial invaders and tissue debris and then attempt to reestablish homeostasis (Clark, 1993). Neutrophils produce inflammatory mediators such as cytokines, chemokines, and free radicals (Cassatella, 1995). Blood derived leukocytes also appear early in subacute phase but in small numbers (Witte et al., 1997). Cytokines produced by astrocytes, microglia, and endothelial cells attract leukocytes and stimulate the production of cell adhesion molecules on leukocytes and

endothelial cells (Feuerstein et al., 1994). If there is reperfusion following an ischemic event, leukocytes are present within one hour and stay at elevated levels for up to five days (Clark et al., 1993). Leukocytes promote the infarction through toxicity of by-products, phagocytic reaction, and immune reaction (Small et al., 1999). Neutrophil activation leads to secondary effects including recruitment and activation of other inflammatory cells, neuronal injury, and glial cell activation (Yong, 1996).

During an ischemic event there is a breakdown of the blood brain barrier (BBB) attributed to trauma of brain vasculature. Trauma-activated endothelial cells and glial cells release free radical species (Wisniewski and Lossinsky, 1991). Cell adhesion molecules are upregulated one to four hours following reperfusion (Small et al., 1999) on the vascular endothelium and at this time serve as a critical docking mechanism for leukocytes to endothelial cells. Neutrophils are chemoattractants for the recruitment of macrophages (Ben-Baruch et al., 1995) which peak at two days post-stroke (Schnell et al., 1999). Macrophages are restricted to the lesion site and operate the removal of tissue debris by phagocytosis (Schnell et al., 1999). The prolonged presence of macrophages can result in other secondary effects. First, their presence can lead to the activation of glia that produce growth factors essential for neuron growth and tissue repair (Lindholm et al., 1990). On the other hand, macrophages can release pro-inflammatory cytokines and/or free radicals, thereby further exacerbating initial tissue damage (Mallat and Chamak, 1994).

iii. Excitability Changes

Within the nervous system, normal function relies on the balance of inhibitory and excitatory operation of individual neurons and pools of neurons. After an ischemic

insult the properties of neuronal activity are altered. Most studies examining changes in cell activity do so seven to ten days post lesion when most of the prominent physiological changes occur (Hagemann et al., 1998). For example, when recording from single neurons within Layer II/III of the cortex, the mean resting potential of an ischemic neuron in the penumbra is lower or less negative than control neurons at seven days (Neumann-Haefelin et al., 1995). Also, at this time, there is an increase in neuronal activity in the peri-lesional area of the cortical infarct.

Increases in neuronal excitability have been linked to changes in receptor density. It has been proposed that increases in excitability are likely due to a down regulation of γ-aminobutyric acid (GABA) receptors resulting in a decrease in GABAergic inhibition of neurons (Neumann-Haefelin et al., 1995). When stimulating Layer IV of the cortex, it has been shown that GABA-mediated synaptic inhibition was weaker (Neumann-Haefelin et al., 1995) and GABA receptor density was decreased (Schiene et al., 1996) in the vicinity of ischemic lesions, leading to the hyperexcitability of single neurons. Not only is there a decrease in GABAergic function but there is also NMDA receptor dependent increased excitability for up to five days in a restricted area around the lesion (Buchkremer-Ratzmann and Witte, 1997). Also, it has been found that the induction of long-term potentiation (LTP) is facilitated in the area around an ischemic infarct (Hagemann et al., 1998). The increase in the tendency of LTP is compatible with the theory that neurons are hyperexcitable and that there is a decrease in GABAergic inhibition observed peri-lesionally. Several processes can be responsible for altering receptor density. Connections directly from the cortex to subcortical structures may be

involved in decreased $GABA_A$ receptor expression and associated hyperexcitability (Schiene et al., 1996).

Another explanation for single cell increases in excitability is alterations in whole cell ion current characteristics. Following an ischemic event, there are two distinct changes in whole cell Ca^{2+} current characteristics. The maximal conductance for Ca^{2+} is enhanced at day seven post lesion and the potential of activation was shifted to more positive potentials (Breuhl et al., 2000). The authors propose that increased ion conduction can be achieved by altering the gating properties or by forming additional channels thereby increasing channel density on neuronal membrane (Breuhl et al., 2000).

The increase in excitability of neurons can have either a beneficial or detrimental effect on the post-ischemic brain. Cells are more excitable, there are changes in the receptor density, and LTP may be facilitated in the areas around the lesion. These modifications may facilitate plastic changes in brain structure (Neumann-Haefelin et al., 1995; Breuhl et al., 2000). Hyperexcitability also has been proposed as the basis for behavioral recovery and compensation following ischemic events (Witte & Freund, 1999). On the other hand, increased excitability may be the foundation for epileptic activity within the injured cortex. Seizure activity is common in human studies with ten to twenty percent of patients experiencing seizures following a stroke (Buchkremer-Ratzmann et al., 1998).

Interestingly, when large populations of neurons are examined there appears to be a decrease in excitability. The field potentials of neurons within the peri-infarct region seem to exhibit a decrease in field potential amplitudes (Neumann-Haefelin et al., 2000). The authors believe that this decrease in overall excitability may be the result of a

decrease in the number of viable neurons or a decrease in the excitability of individual neurons or possibly both. Neumann-Haefelin and colleagues (2000) propose that the decrease in the number of functional neurons may outweigh the increase in excitability of single neurons.

Chronic Phase (10 Days +)

The chronic phase of stroke includes ten days post-ischemia and all time points after. The infarct has been formed and most neuronal functional outcomes have been established by the chronic phase. Some neuronal characteristics altered in the acute and subacute phases continue to persist.

i. Persistent Changes in Neuronal Characteristics

Some of the functional neuronal alterations associated with the acute and subacute phases of stroke seem to return to normal over time. For example, permeability disturbances in the blood brain barrier (BBB) which occur post-ischemia are reversed at ten days post lesion (Schnell et al., 1999). However, some functional changes within the peri-infarct region or in remote areas persist for longer than one month (Neumann-Haefelin et al., 2000). $GABA_A$ receptor density, which is initially upregulated post-ischemia, is lower than normal within the chronic phase (Schiene et al., 1996; Que et al., 1999). The persistent receptor density changes may cause the preserved decreased inhibition at two months (Buchkremer-Ratzmann et al., 1997). Another enduring change is the maximal conductance of Ca^{2+} channels which lasts at least one month (Breuhl et al., 2000). Metabolic disturbances also seem to persist into the chronic phase. The rate of regional oxygen metabolism within the infarct remains depressed which implies that there is no delayed recovery of mitochondrial function (Wise et al., 1983). Metabolism

of glucose is decreased in exofocal regions within the chronic phase (Nagasawa et al., 1994). Considering all of the persistent remote neuronal changes, it is surprising that the number of apoptotic cells in remote regions returns to normal by two months post-ischemic insult (Contil et al., 1998).

BEHAVIORAL DEFICITS ASSOCIATED WITH ISCHEMIA

The neuropathology associated with the cellular consequences of stroke also produce persistent behavioral deficits. To assess impairment severity following ischemia clinicians use measures such as the National Institute of Health Stroke Scale (NIHSS) based on neurological examination of sensory, motor, reflex, and language functions often disrupted after stroke (Heinemann et al., 1997). Specifically, the NIHSS evaluates the level of consciousness, visual abilities, motor function of the face, arms, and legs, sensory deficits of all limbs, language skills, and the presence of neglect (Williams et al., 2000). Studies indicate that this scale is the most accurate in moderate ranges of impairment but tends to either over or underestimate deficits with low or high ratings of impairment (Heinemann et al., 1997; DeGraba et al., 1999).

Because not every ischemic event is identical, the behavioral impairments vary. During the acute phase of stroke, the Heart and Stroke Foundation lists a number of warning signs that occur almost immediately after an ischemic event has occurred. Such warning signs include weakness in the face, arms, or legs, loss or inability to understand speech, visual deficits, severe and unusual headache, or sudden loss of balance usually associated with the other symptoms. Recent studies confirm the occurrence of decreased strength in upper extremities (Cramer et al., 1997) or facial palsy within 24 hours of stroke (Williams et al., 2000). There is also evidence of spontaneous recovery in

approximately twenty percent of patients in the acute phase (Toni et al., 1997). The Canadian Neurological Scale (CNS) provides evidence that with transient ischemic attacks neurological deficits may resolve within twenty-four hours (Cote et al., 1986).

In the subacute phase, even though behavioral impairments persist this is the phase in which recovery is most rapid (Wade et al., 1985). Evaluating patients with the CNS shows that during the subacute phase eighty-four percent of patients exhibit at least one motor deficit (Cote et al., 1986). There seems to be a clinical syndrome present during this phase in which patients will most likely experience muscular weakness, absence of spontaneous movements, impaired movement initiation and slowness, and problems holding constant force (Classen et al., 1997). Specifically, this study investigated the movements and force produced in the affected upper extremity. Patients were required to tap the index finger at their preferred speed and keep the pace as regular as possible. They were also required to abduct the index finger against resistance and keep the force as steady as possible. They found that patients exhibited slow, clumsy, and awkward tapping and alternate finger movements. They had difficulties in initiating activity of target muscles and starting isometric contractions. The ability to maintain isometric contractions was also impaired. In another study, half of the stroke patients required aid in tasks such as walking, dressing, and feeding (Wade et al., 1985). The investigators recorded the function of patients in these tasks for the first thirteen weeks after they experienced a stroke. They found that the greatest amount of recovery occurred within the first two weeks continuing slowly for the ninety days of the study, however, the majority of patients exhibited some sort of disability in the functions listed above.

Behavioral recovery at some level will continue into the chronic phase of stroke. At three months eighty percent of patients will become independent on tasks such as walking, dressing, and feeding (Wade et al., 1985). However, there are behavioral impairments that will persist for much longer periods of time. Hemiparesis, the partial loss of movement or sensation in the affected side of the body, may be present at six months (Macko et al., 2001), one year (van der Lee et al., 1999), or even five years post stroke (Miltner et al., 1999). For example, misdirected multi-joint arm movements appeared when patients were required to move an upper limb towards a target (Beer et al., 2000) one year post-stroke. Less accurate arm movements were exhibited when patients are required to perform a random sequence of movements in an unpredictable situation an average of four years following damage (Velicki et al., 2000). These movement disorders become visible in a laboratory situation when patients are required to perform specific movements with their affected extremities. Stroke patients exhibiting these behavioral impairments in most cases are able to perform daily tasks independently. Although behavioral impairments may persist for extended periods of time, there seems to be some level of recovery that continues past the acute phase (Wade et al., 1985).

It is almost certain that some sort of behavioral impairment will remain because it is unlikely that an individual will fully recover following a stroke. There is a difficulty associated with the definition of "true" recovery versus functional and behavioral compensation. Recovery has two components. The first, restitution, results from a return to normal function by the injured brain. The second is substitution, in which there is a redistribution of function where other brain areas "take over" the performance of the disabled function (Geldmacher, 1997). An additional component of substitution is

compensation. Compensation does not represent "true" recovery because the brain substitutes areas for others to make up for deficiencies. For example, it is more likely that a stroke patient will learn to walk with the aid of a cane than recover the ability to return to a normal gait. In addition, when stroke patients are trained on a reaching task they develop compensatory strategies different from control subjects (Cirstea and Levin, 2000). For example, stroke patients reached the target with a lack of continuity utilizing a series of small sequential movements instead of smooth, continuous movements exhibited by healthy subjects (Cirstea et al., 2000). Also, following experimentally induced strokes rats develop stereotypical behavioral patterns different from those they used before ischemia (Friel and Nudo, 1998) such as different strategies in aiming and postural adjustments when reaching (Whishaw, 2000). Therefore, it seems that stroke patients are more likely to find a way to compensate for their deficit rather than truly recover.

REHABILITATION

To minimize the impairments exhibited following the occurrence of a stroke rehabilitative therapies have been developed. The World Health Organization defines stroke rehabilitation as a progressive, dynamic, goal-oriented process aimed at enabling a person with an impairment to reach his or her optimal physical, cognitive, emotional, communicative, and/or social functional level. The ultimate goal of rehabilitation is to improve the quality of life for stroke survivors and their caregivers (Phillips et al., 2002). This goal can be achieved through many processes. For example, therapy has been defined as the "process of treatments and experiences, prescribed by clinicians and conducted in collaboration with patients and their families, that promotes, facilitates, or

encourages the patient's rehabilitation" (Geldmacher, 1997). Identifying which treatments and experiences are more beneficial for an individual stroke patient can be problematic. Each ischemic event causes a different array of impairments for stroke patients, thus specific rehabilitation strategies need to be developed to meet individual needs. The Acute Stroke Unit at the Queen Elizabeth II Health Sciences Centre has developed a rehabilitation strategy of organized care attempting to fulfill this requirement (Phillips et al., 2002). The multidisciplinary team includes physiotherapists whose duty is to assess and treat motor impairments including motor control, strength and physical conditioning, balance, and gait and mobility training. Social workers are also involved in emotional and adjustment counseling for patients and families. Speech-language pathologists diagnose and treat communication disturbances or swallowing disorders. Similar studies have shown that the care of stroke patients by a coordinated multidisciplinary team is effective in reducing mortality and morbidity (Hankey and Warlow, 1999; Indredavik et al., 1999).

Research is critical for evaluating new rehabilitation techniques, developing effective evidence-based approaches to stroke rehabilitation, and for making system improvements. Specific areas of research include studying the treatment of sensorimotor deficits and impaired mobility assessment on the efficacy of exercise and functional motor training. This foundation believes that there is a need for more rigorous testing of new techniques such as constraint-induced paradigms of rehabilitative therapies. Such experiments are currently being conducted on animals such as the rodent (Miltner et al., 1999; van der Lee et al., 1999; Bland et al., 2000). In this thesis rehabilitation, can be

defined as the training on a skilled forelimb motor task that promotes, facilitates, and encourages the use of forelimb movements impaired following an ischemic event.

II. DIASCHISIS

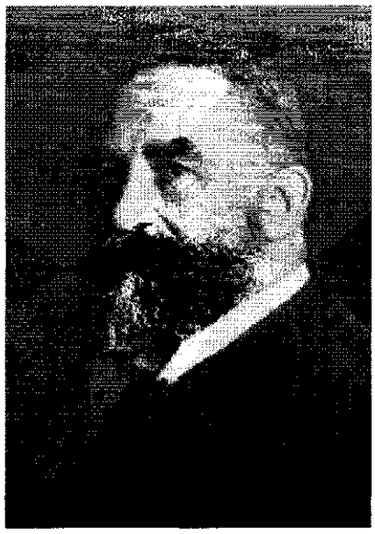

Figure 1.3- Constantine von Monakow (1853-1930) coined the term "diaschisis".

EARLY RESEARCH

In neuroscience research, investigators try to link underlying neural mechanisms with behavioral performance. With each ischemic event, there are associated behavioral impairments. Almost one hundred years ago von Monakow coined the term 'diaschisis' to explain initial behavioral symptoms in clinical cases that could not be accounted for by the size of the structural lesion itself (von Monakow, 1914). Purely from observations of patients, he hypothesized that diaschisis represented a sudden interruption of function originating from a focal lesion with an abolition of excitability that reaches remote areas of the brain and spreads as a form of passive inhibition (von Monakow, 1914). Damage to one area produced cessation of function in regions connected to the primary site of injury. Much of the current work examining the neural mechanisms underlying ischemic cell death has focused on the infarct core (Kristian et al., 1998; Small et al., 1999).

However, there is now considerable evidence that many of the impairments and subsequent recovery may depend upon changes within peri-lesional regions (Stroemer et al., 1995; Dirnagl et al., 1999; Vila et al., 2000) supporting von Monakow's hypothesis. Therefore, behavioral impairments may be exaggerated in relation to the actual size of the lesion. If interruption of function outside the lesion core can be resolved, it may then lead to behavioral recovery.

von Monakow proposed three types of diaschisis: cortico-spinalis, commissuralis, and associative diaschisis. First, cortico-spinalis diaschisis results from the progression of functional depression from primary motor cortex injury to the spinal cord along pyramidal tract fibers (Feeney and Baron, 1986). Commissuralis diaschisis results from depression crossing to the contralateral cortex via axons of the corpus callosum. Finally, associative diaschisis is intracortical fiber-mediated depression of function in intact cortical areas neighboring the locus of injury (Feeney et al., 1986).

Beginning in the 1950s, Kempinsky confirmed that the neural dysfunction associated with diaschisis depends on the internal organization of the nervous system and its afferents (Kempinsky, 1958). He focused on the cerebral hemisphere contralateral to ischemic injury and found that transient depressions of cortical electrical activity are dependent on an intact corpus callosum. He concluded that the activity of a neuronal group is facilitated by a constant input of impulses from other neuronal groups. When the facilitator group is destroyed, the dependent neuron groups will become less active. Kempinsky (1958) clarified that diaschitic inhibition is an active inhibitory process resulting from a withdrawal of facilitation of neuronal afferents communicated via the corpus callosum.

In the 1960s, researchers began to explore changes of cerebral blood flow (CBF) in areas surrounding and remote from the lesion core. A number of studies revealed that there were decreases of CBF in the contralateral hemisphere (Waltz et al., 1966) as well as in intact regions of the ipsilateral hemisphere (Skinhoj, 1965) following an ischemic insult. Continuing his work, Kempinsky proposed essential criteria for diaschisis. He stated it must be a circumscribed injury, have a neuronal basis for depressive effects, occur at least one synapse from injury, the fiber tract responsible must be identified, and it must be reversible (Kempinsky, 1966).

Studies in the 1970s began investigating the characteristics of diaschisis and its causes. Studies in human patients revealed that the likelihood of diaschisis might differ between ischemic events. Some reports indicate between fifty (Fujishima et al., 1974) and one hundred percent of patients experience diaschisis (Melamed et al., 1975). At this time, the definition of diaschisis was expanded to include symptoms of metabolic or circulatory dysfunction. CBF seems to vary with severity of infarct (Meyer et al., 1971), the reduction seems to peak at one week post lesion (Slater et al., 1977), and the decrease in both hemispheres seems to persist for about three weeks post stroke (Meyer et al., 1979). Reductions in CBF persist only in the ischemic hemisphere and flow values return to normal in homologous zones in the opposite hemisphere (Meyer et al., 1979).

Simultaneous elevated levels of glucose and lactate occur with reduced CBF resulting in decreased levels of ATP (Levy and Duffy, 1977). Also, tissue immediately around the infarct region seems to suffer a temporary loss of function and regains its original excitability during the course of behavioral recovery (Glassman, 1971). Some investigators have proposed that reduced CBF in the contralateral hemisphere was due to

a transhemispheric steal brought about by redistribution of CBF that 'steals' blood from the contralateral hemisphere to provide collateral circulation to ischemic regions (Zulch and Eschback, 1972). Contralateral regions of the brain may also become ischemic by acting as collaterals of a major vessel (Zulch et al., 1972).

von Monakow began diaschisis research as a hypothesis to explain exaggerated behavioral abnormalities of patients with brain injury. He classified diaschisis based on his hypothesis. By the 1970s, it was shown that diaschisis does depend on the internal organization and the depression of facilitator neuronal groups. Also, at this time, it had been shown that factors such as CBF and the type of ischemic event influence the occurrence of diaschisis. More recent research has attempted to elucidate the mechanisms by which diaschisis occurs.

RECENT RESEARCH

In the 1980s, an increasing amount of research investigated the characteristics and mechanisms of diaschisis. Using new techniques such as positron emission tomography (PET) investigators continued to evaluate oxygen use, glucose metabolism, and cerebral blood flow (CBF). Researchers were able to match a depression of blood flow and metabolism to the area of the lesion but there was a normal level of oxygen extraction in the cerebellar hemisphere contralateral to the infarct (Baron et al., 1980). Subsequent studies confirmed diaschisis following cerebral hemisphere infarction present in the cerebellum (Lenzi et al., 1982; Martin and Raichle, 1983; Naritomi, 1983), in other subcortical structures such as the basal ganglia, red nucleus, inferior olive, (Dauth et al., 1980; Dauth et al., 1985) and the thalamus (Kuhl et al., 1980; Heiss et al., 1983).

A controversy regarding the duration of diaschisis in the form of hypometabolism and hypoperfusion began in the 1980s. Baron and colleagues (1980) reported diaschisis was present only in the acute and subacute phases of stroke. In the contralateral hemisphere, regional CBF seemed to return to normal or remained minimally reduced by the third or fourth week following lesion (Meyer et al., 1987). However, crossed cerebellar diaschisis was found to persist for as long as fifty days (Lenzi et al., 1982), six months (Meneghetti et al., 1984), or two years post stroke (Martin et al., 1983). A few studies found that with a larger and parietally situated infarction there was a greater extent of cerebellar diaschisis (Lenzi et al., 1982; Kushner et al., 1984). However, Martin and others (1983) reported more marked cerebellar diaschisis following anterior lesions. Therefore, diaschitic dysfunction seems to depend on factors such as the location and size of ischemic injury.

Theories regarding the cause of diaschisis continued to be proposed. Most of the research examined two types of remote effects in the contralateral hemisphere. The first was diffuse depression of metabolism and blood flow through the entire hemisphere. The other was CBF and metabolic depression located within a homologous cortical locus (Meyer et al., 1987). A division between acute and subacute or chronic phases of stroke related to their affect on diaschisis was also being distinguished. For example, processes occurring during acute and subacute phases were linked to diaschitic dysfunction such as depression of the ipsilateral cortex and was thought to be due to edema (Meyer et al., 1987), increased intracranial pressure (Beaney et al., 1985), diffusion of toxic waste products from the core of the infarct, or selective neuronal death (Feeney et al., 1986). Water content, sodium and potassium ratios, or intracellular and extracellular pH were

proposed as possible causes of diaschisis (Meyer et al., 1987). Kempinsky (1966) expanded on his theory of how diaschisis is caused by loss of facilitation. He proposed the dependent neuronal groups become less active but eventually become independent and then begin to function at their initial level facilitating recovery (Kempinsky, 1966).

In the years following this research, diaschisis was separated into four categories of nonischemic changes in areas immediately surrounding the lesion or in remote brain areas. The first category is the remote changes caused by edema. In models of middle cerebral artery occlusion (MCAO), if there was a severe ischemic event and the infarction was large, brain edema developed in remote, nonischemic areas of the brain (Meyer et al., 1987). Tissue swelling may produce massive secondary damage by compression of the contralateral brain and other areas. The damage was usually present within three days of ischemic insult (Witte et al., 2000). A second category involves functional changes possibly caused by waves of spreading depressions present in the subacute phase of stroke. These depolarizations are not constrained to areas of infarction but travel across normal tissue (Witte et al., 2000). Buchkremer-Ratzmann and Witte (1997) found that using MK-801, a NMDA receptor antagonist that may block spreading depressions, did not decrease infarct size when diaschisis was present. They proposed that the presence of electrophysiological diaschisis was not due to the spreading depression emanating from the lesion. Also, the effects of spreading depressions did not last longer than one week (Witte et al., 2000) and diaschitic dysfunction has been known to persist for much longer. Third, diaschisis may be due to changes in projection areas due to necrosis, deafferentiation, or degeneration. Diaschisis may be due to an undercutting process as a result of necrosis of subcortical white matter isolating the overlying cortex from afferent

input and severed efferent axons (Feeney et al., 1986). The presence of thalamic hypometabolism following cortical ablation may reflect retrograde degeneration (Feeney et al., 1986). PET and computed tomography (CT) scan studies report that disconnections do occur between regions, which show secondary reductions of regional CBF and metabolism (Meyer et al., 1987). Others have reported that damage to thalamus and thalamocortical projection systems result in ipsilateral cortical hypometabolism producing neuropsychological impairments (Pappata et al., 1990). Crossed cerebellar diaschisis has been linked to anterograde degeneration of the corticoponotocerebellar system (Yamauchi et al., 1992). Continuous decreases in glucose metabolism may reflect irreversible neuronal dysfunction resulting from retrograde or anterograde degeneration or transneuronal cell damage at the chronic stage (Nagasawa et al., 1994). The diaschitic effects post-lesion follow the extension of collaterals from the lesion site suggesting deafferentiation and degeneration of collaterals (Witte et al., 2000). Finally, diaschisis may also be caused by changes in reactive plasticity and systemic effects (Schiene et al., 1996). Reactive plasticity is associated with changes in inhibition or depression and may facilitate recovery from an ischemic infarct. Some authors propose that this peri-lesional dysfunction may have a positive effect (Witte et al., 1999).

FUNCTIONAL RECOVERY & DIASCHISIS

Resolution from diaschisis has been proposed as the event responsible for behavioral recovery following stroke. This theory is difficult to prove because diaschisis is difficult to quantify. Many studies attempt to utilize cerebral blood flow (CBF), oxygen extraction (Baron et al., 1980), electrical activity (Buckremer-Ratzmann et al., 1997), or cellular metabolism (Ginsberg et al., 1989) as indirect measures of diaschisis.

Following resolution of these indirect measures of diaschisis there is recovery of behavioral deficits. In human subjects, the severity and outcome of the neurological deficit was correlated with blood flow and metabolism in the injured hemisphere (Meyer et al., 1971). A low level of regional CBF has been implicated in a poor clinical outcome (Lenzi et al., 1982). Also, glucose metabolism has been suggested as a multi-focal brain dysfunction caused by neuronal network disturbances which may exacerbate the clinical symptoms following stroke (Nagasawa et al., 1994).

It is certain that structural, functional, and behavioral alterations result from an ischemic event. However, the exact functional impairments following these changes are unknown. Diaschisis has been proposed to demonstrate how the structure and function of the nervous system controls behavior. It is possible that diaschisis may explain how behavioral manifestations seem to recover over time despite persistent structural and functional aberrations. At this time, diaschisis encompasses all remote effects of an injury. A more specific definition describes diaschisis as the "initial stage of temporary flaccid weakness and depressed consciousness which can appear immediately following localized infarction within one cerebral hemisphere" (Kempinsky, 1966). It is impossible to investigate all the structural, functional, and behavioral aberrations following an ischemic event. Thus, the focus of this thesis will be changes within the motor cortex immediately surrounding ischemic tissue associated with specific behavioral alterations, subsequent functional recovery, and the resolution of diaschisis.

III. MECHANISMS OF RECOVERY

Although the cortex is statically organized into layers and intrinsic horizontal and vertical connections, there are dynamic characteristics of the cortex. Plasticity in this context refers to the reorganization of movement representations within the motor cortex in response to environmental manipulations. These dynamic properties result in motor map plasticity providing the substrate for recovery following an ischemic event. Mechanisms have been proposed as the means by which the topography of the motor map is altered in relation to environmental factors. First is the growth or collateral sprouting of new connections. On the other hand, alterations in the effectiveness of existing connections by forming new synaptic contacts or potentiating existing synapses (Kaas, 1991) may result in plasticity of the motor cortex. The intrinsic connections of the cortex have plastic properties which facilitate map reorganization. These connections have also been proposed as the substrate for the method by which rehabilitation facilitates the resolution from diaschisis. The system of connections functionally associates neurons to form assemblies that construct dynamic maps (Sanes and Donoghue, 2000). Insight into the processes by which reorganization supports recovery from ischemia have been taken from studies of learning, abnormal experience, and changes to map topography following lesions.

LEARNING

Underlying neurophysiological alterations responsible for behavioral recovery may be the same as those mediating motor learning (Lee and van Donkelaar, 1995). Following cortical damage, an organism must relearn to perform actions with the affected part of the body. The topography of movement representations within primary motor

cortex has been shown to alter following the acquisition of a motor skill (Nudo et al., 1996b; Kleim et al., 1998). Motor skill learning is the acquisition of new spatiotemporal muscle-activation patterns in such a way that individually known movements combine to form a novel movement sequence (Sanes et al., 2000).

Motor representational changes, as a result of motor skill learning, can be seen in humans and in animals. For example, piano players show increased activation in the primary motor cortex associated with more rapid skill acquisition as compared to non-musicians (Hund-Georgiadis and von Cramon, 1999). This is consistent with other studies showing increased activation when a person learns a new task, such as repetitive finger movements in fMRI (Karni et al., 1995), positron emission tomography (PET) (Schlaug et al., 1994), and transcranial magnetic stimulation (TMS) studies (Classen et al., 1998). Experienced racket ball players compared to novices or non-players, in studies using TMS, experience functional reorganization such as increased corticomotor excitability and a spatial shift in movement representations of the playing hand associated with the acquisition and retention of complex motor skills (Pearce et al., 2000). There have even been reports of physiotherapy producing a use-dependent enlargement of motor cortex representations (Liepert et al., 2000). Stroke patients enrolled in rehabilitation also exhibit similar changes in cortical representations, demonstrating that the development of motor recovery can reflect learning. For example, in fMRI (Cramer et al., 1997) and PET (Weiller et al., 1993) studies, patients exhibit increased bihemispheric activation when asked to do tasks with the recovered hand. As highlighted in the previous section, a decrease in activation as a patient recovers is associated with

the increase in synchronized action of motor circuits resulting in a increase in corticospinal output (Liepert et al., 2000).

In animals, similar changes to motor representations following learning have been found. Researchers have found that use-dependent changes in cortical representations occur in cats (Kimura et al., 1996) and squirrel monkeys (Nudo et al., 1996b). Specific training paradigms involving reaching and retrieval tasks in squirrel monkeys (Plautz et al., 2000) and in rats (Kleim et al., 1998) have been shown to produce a shift in movement representations to movements which are involved in the performance of the task, however, motor reorganization seems to be a very task specific (Suner et al., 1993; Plautz et al., 2000). Strength training does not alter forelimb movement representations in rats when compared to a similar skilled training paradigm (Remple et al., 2001). A voluntary exercise paradigm in which rats are allowed access to a running wheel does not influence movement topography but does induce angiogenesis (Kleim et al., 2002). In rehabilitation models, recovery also seems to be task specific. When rats are trained on skilled or unskilled reaching tasks following a focal ischemic infarct, functional organization and behavioral performance for the skilled rehabilitation condition resemble pre-lesion levels (Goertzen et al., 2001). Unskilled rehabilitation resulted in motor map topography and reaching performance that resembled animals which did not receive rehabilitation at all. Reorganization of motor cortex movement representations has been linked to synaptogenesis (Kleim et al., 1996; Kleim et al., 2001) and increases in synaptic efficacy (Sakamoto et al., 1987) accompanying skill learning (Rioult-Pedotti et al., 1998). In peri-infarct tissue, skilled rehabilitation retains more excitatory synapses per neuron, while there is a significant decrease in synapses per neuron in unskilled rehabilitation and

non-rehabilitation conditions (Goertzen et al., 2001). These increases in synapses may be on intracortical afferents and may facilitate cortical reorganization and recovery.

ABNORMAL EXPERIENCE

A second form of experiential influence on the motor map topography of movement representations related to recovery following ischemia are changes in peripheral experience. Like learning, environmental influences manipulate motor processing within the motor cortex. Manipulations include amputations, repetitive stimulation, peripheral nerve lesions, and tactile experience.

Amputations result in changes of the topography of movement representations. In humans, following limb amputation maps of outputs were larger for muscles ipsilateral to the amputation as compared with the contralateral side (Cohen et al., 1991; Karl et al., 2001). Not only are representations larger but thresholds required to elicit a response are lower on the side of the amputation (Donoghue and Sanes, 1988; Hall et al., 1990). Amputation results in a shift of cortical devotion from areas normally represented to adjoining body parts in monkeys (Wu and Kaas, 1999; Qi et al., 2000) and rats (Donoghue and Sanes, 1987; Donoghue et al., 1988). Similar results from motor nerve lesions result in reorganization within hours of transection (Sanes et al., 1988; Donoghue et al., 1990) and persist for longer periods of time (Sanes et al., 1989). Plasticity also allows for a reversal of this phenomenon. A patient received bilateral hand transplantation, which reversed amputation-induced cortical reorganization (Giraux et al., 2001). The increases in motor outputs and lowered cortical thresholds suggest that there is damage-induced excitability in the cortex.

Repetitive cortical stimulation can also alter movement representations in the motor cortex. In humans, repetitive transcranial magnetic stimulation (rTMS) results in a gradual increase in the size of representation (Berardelli et al., 1998). Repetitive intracortical microstimulation (ICMS) produces the same result in rats (Nudo et al., 1990). Following repeated peripheral nerve stimulation in humans, there is also an expansion of representational maps of stimulated muscles (Ridding et al., 2001). In a different paradigm, induction of repeated seizure activity or kindling, is accompanied by increased synaptic strength resulting in doubling of size of the CFA of rat motor cortex (Teskey et al., 2001).

Amputations (Dettmers et al., 2001) and repetitive stimulation (Nudo et al., 1990) seem to alter the balance of excitable and inhibitory influences within the cortex. Similarly, ischemia causes a marked increase in excitability (Dirnagl et al., 1999; Small et al., 1999; Zipfel et al., 2000) and decrease in GABAergic inhibition (Neumann-Haefelin et al., 1995; Schiene et al., 1996). Functional compensation is related to the normalization of this shift in excitability. With a decrease in inhibition the nervous system strengthens connected cells and this may underlie modifications in motor cortical output (Sanes and Donoghue, 1997). Therefore, the cortical environment after amputation or repetitive stimulation, is similar to the effects of ischemia.

Changes in tactile information can be in the form of whisker trimming or plucking, postural adjustments, immobilization, or manual tactile stimulation. Following whisker plucking or trimming, there is a significant reduction in the area of vibrissae representations (Keller et al., 1996; Huntley, 1997; Kossut and Juliano, 1999). If vibrissae are allowed to regrow, representations return to normal (Keller et al., 1996).

Ipsilateral representations of vibrissae are not altered following trimming (Huntley, 1997). Changing the posture of the forelimb in rats had either immediate or delayed expansion of forelimb representation into vibrissae areas depending on the type of posture (Sanes et al., 1992). In human patients, immobilization of a joint without a peripheral nerve lesion, caused the motor cortex representation of the inactivated muscles to diminish as compared to the unaffected leg (Liepert et al., 1995). This effect was quickly reversed by voluntary muscle contraction. Manual tactile stimulation of an area of the throat in humans results in increased excitability and area of representation (Hamdy et al., 1998). Cortical representations may then be dependent on certain levels of excitability induced by movement. Losses in representations may be due to ischemia-related non-use (Kopp et al., 1999) or changes in tactile information such as vibrissae removal.

LESION EFFECTS

Not only can learning and abnormal environmental influences alter the topography of the motor map, but cortical and subcortical lesions can as well. In humans, after resection of a tumor adjacent to primary motor cortex, additional primary motor cortex representations appear (Duffau, 2001). Patients with lesions of the posterior area of the internal capsule exhibit an extension of the hand field in the contralateral motor cortex (Weiller et al., 1993). Tumors within the cortex also cause shifting of motor representations (Wunderlich et al., 1998). In the rat, bilateral frontal cortex lesions on postnatal day four cause displacement of forelimb representations to areas of non-motor parietal cortex (Kleim et al., 2001). Cerebellar lesions can increase the threshold required to elicit evoked movements with ICMS (O'Donoghue et al., 1986).

RECOVERY OR COMPENSATION?

The majority of the results discussed thus far have highlighted circumstances in which recovery is possible following an ischemic event. What exactly constitutes recovery? The Webster's Encyclopedic Dictionary (1988) defines recovery as "the act of recovering or to bring back to normal condition." Following an ischemic event there are many behavioral and functional impairments within the motor system. Three outcomes have been proposed following brain injury. First, there is compensation resulting from an adaptation in which an organism changes its strategy or the substitution of a new behavior for a lost one (Kolb, 1995). Second, there is partial restitution of the original behavior which reflects recovery from some sort of nonspecific injury or a genuine return of function. Finally, there can be a complete restitution of the original behavior (Kolb, 1995). Previous research has shown however that rarely does an organism return to normal following ischemia (Friel et al., 1998; Cirstea et al., 2000; Whishaw, 2000). Careful evaluation of recovery indicates that the most likely outcome is partial recovery of function along with substitution of function (Kolb, 1995).

Compensation is defined as the ability to achieve a goal by substituting remaining movement abilities for lost movements (Whishaw, 2000). It has been proposed that plasticity following brain injury is the result of two interacting variables. First, the cortex can be altered functionally based on specific motor experiences in which new motor skills are acquired and resulting compensatory alterations in physiological and anatomical organization ensue (Nudo, 1999). Second, these compensatory changes occur in the functional organization of uninjured adjacent tissue (Nudo, 1999). In other words 'recovery' of function may reflect the action of healthy tissue or function may be restored

by undamaged tissue (Hier, 1986). Recovery of behavior may not be due to spared neurons assuming the functions of lost neurons, but may be due to spared neurons changing morphology to support compensatory skills (Kolb and Whishaw, 1998). For example, when stroke patients attempt to move but experience deficits in behavior, the natural reaction is to compensate with available and successful motor strategies (Cirstea et al., 2000). The underlying neurophysiological modifications responsible for behavioral changes may be the same for motor learning and for recovery of motor skills following brain injury (Lee et al., 1995).

IV. THE PROBLEM OF MEASURING RECOVERY & ALLEVIATION OF DIASCHISIS

The role that overcoming diaschisis plays in recovery is poorly understood. Several characteristics are required to study the relationship between diaschisis, behavioral deficits, and recovery. First, a brain region whose function can be reliably defined is needed. Second, the function of that region and behavior must have a clear relationship. Third, if damage occurs in this defined cortical area there must be behavioral deficits. The level of impairment and recovery must be defined and assessed. Fourth, recovery must be modifiable through rehabilitative interventions.

RODENT MOTOR CORTEX AS A MODEL

The rodent provides a model that meets the criteria needed to investigate the role of diaschisis in behavioral recovery. First, rats posses a highly developed motor system that supports an extensive behavioral repertoire. Central to this system is the motor cortex. The rodent motor cortex provides an ideal model because the structural and functional organization of the motor cortex is not unlike that of the primate motor cortex making it possible to compare results to the human motor system. Further, a considerable amount of work has been done to describe the intrinsic organization of the rat motor cortex and the relationship between the organization of motor behavior and its role in controlling movement. The functional organization of the rat motor cortex has been accurately and reliably defined using intracortical microstimulation (ICMS) (Neafsey et al., 1986; Kleim et al., 1998). The use of ICMS provides the technique to measure the relationship between the brain and behavior.

Furthermore, damage to physiologically defined areas of the motor cortex produce motor deficits. Specific components of normal motor skills and motor impairments following damage to the motor cortex have been analyzed in detail. When using a single pellet reaching task specific components of the behavior can be measured (Whishaw and Pellis, 1990) and deficits are observable following motor cortex damage (Whishaw and Kolb, 1988). More importantly, recovery can be observed with training (Goertzen et al., 2001). The characteristics of the rodent motor cortex provide a model in which the direct effects of ischemia on the motor cortex including diaschisis, the resulting behavioral deficits, and functional recovery can be studied extensively.

THE RAT MOTOR CORTEX

The rat motor cortex organization provides the substrate for a model by which to study diaschisis and its relationship to behavioral impairments and recovery following stroke. The anatomical organization includes the pattern of connections between neurons and how they interact to produce overt movement patterns. Functional organization within the rat motor cortex contains topographic representations of the rat's body. It is important to explain anatomical and functional organization of the rat motor cortex to understand how ICMS is used to measure the presence of diaschisis following an ischemic event.

i. Anatomical Organization

The intrinsic organization of the motor cortex is organized by connections between neighboring neurons (Figure 1.4). Layers of the motor cortex are connected by extensive corticocortical connections to coordinate limb movements (Porter and Lemon, 1995). Neurons within layer V project via horizontal axon collaterals for long distances

within layers III and V (Aroniadou and Keller, 1993). The long, horizontal axon collaterals provide the substrate for synaptic interactions among functionally related neurons (Huntley and Jones, 1991). These cells participate in feedback pathways for inhibiting and increasing excitability of corticospinal neurons (Landry et al., 1980). Pyramidal tract neurons within layer V have horizontal collaterals which project within layers V and VI (Landry et al., 1980). The axon collaterals of layer V pyramidal neurons have an extensive terminal field including layers II through V (Keller, 1993).

The motor cortex utilizes both the horizontal and vertical organization to produce movement. Activation starts with impulses arriving at the motor cortex from a variety of fibers which then activate cortical interneurons in the superficial and intermediate input layers with simultaneous activation of corticofugal fibers from specific inputs (Asanuma and Rosen, 1973). These activated interneurons inhibit or excite other neurons by way of columnar connections extending along radial fibers. When the impulses reach the deep layers, they are initiated and sent back to neurons in superficial and intermediate layers constituting a feedback loop within the cerebral cortex (Asanuma et al., 1973). There have been discrete neuron colonies reported within the motor cortex of cats (Asanuma and Sakata, 1967) and monkeys (Asanuma and Rosen, 1972). Each neuron colony extends along radial fibers within gray matter and constitutes a columnar structure or a cortical efferent zone (Asanuma et al., 1973). The efferent zone receives inputs mostly from the region located at the distal portion of muscle to which the efferent zone projects (Asanuma et al., 1973). Investigators report output columns, which contribute to the same movement, are clustered together and form efferent zones (Gu et al., 1999).

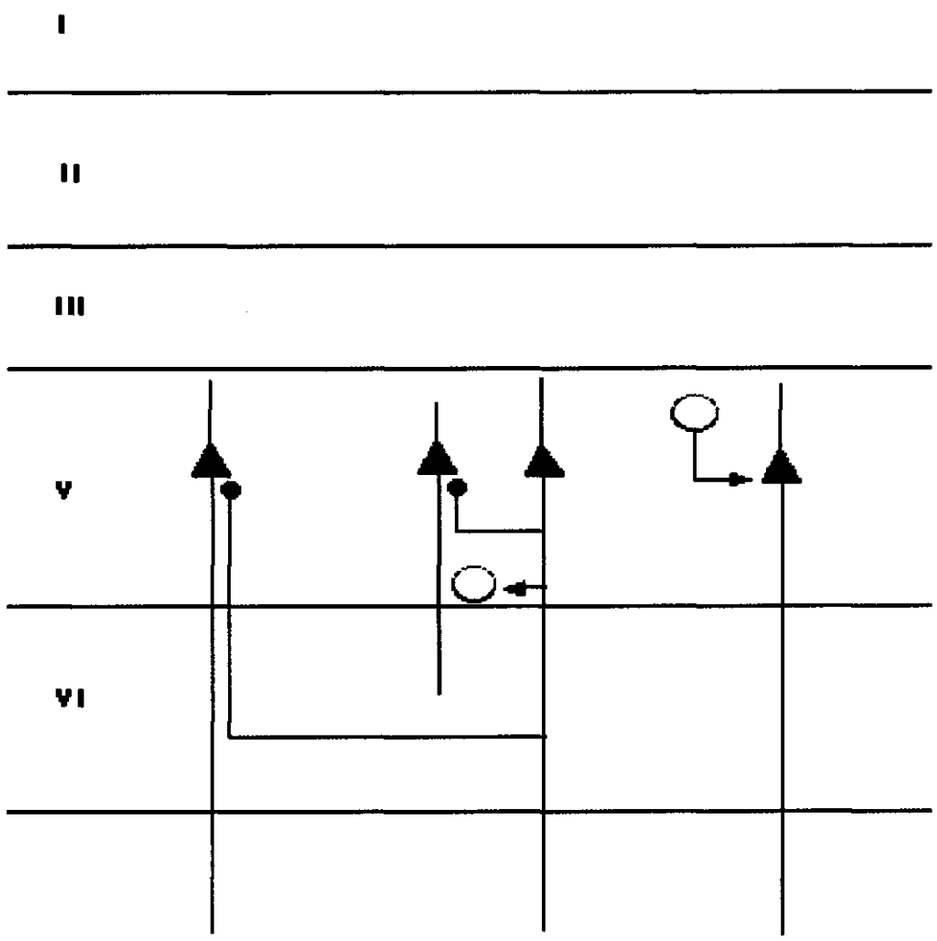

Figure 1.4 - Intrinsic circuitry of the motor cortex. Layer V of the motor cortex contains both pyramidal cells (blue triangle) and non-pyramidal interneurons (open circle). Intracortical connections can be excitatory (red circle) or inhibitory (black triangle). Stimulation of Layer V activates excitatory cortical connections between pyramidal cells as well as inhibitory interneurons.

ii. Functional Organization

The rat motor cortex contains a detailed map of topographic movement representations. The topography of forelimb representations within the rat motor cortex can be constructed with the use of intracortical microstimulation (ICMS). Topographic representations within the rat motor cortex are physiologically defined areas that produce

overt movement responses when the cortex is stimulated. Using standard ICMS techniques (Kleim et al., 1998; Remple et al., 2001) the rat is anesthetized and a craniotomy is performed over the motor cortex contralateral to the preferred paw. Following this preparation, a microelectrode controlled by a hydraulic microdrive is positioned at various locations on the cortex and then lowered into layer V. A small amount of current is passed through layer V stimulating pools of pyramidal cells and the subsequent evoked movement pattern is recorded. After repeated microelectrode penetrations are made across the cortex, the end result is a topographic map of representations within the motor cortex.

The motor cortex of the rat contains a somatotopic representation of the rat body. It is characterized by a large forelimb area located rostral and lateral to the hindlimb representations. The trunk representations posteriorly overlap the hindlimb area. Vibrissae representations are located medially and overlap the forelimb, trunk, anterior part of the hindlimb areas, and neck areas. Jaw and tongue representations are located anterolaterally and the tail area is located in the median area of the hindlimb representation (Settlage et al., 1949; Hall and Lindholm, 1974; Donoghue and Wise, 1982; Gioanni and Lamarche, 1985; Neafsey et al., 1986). In addition to these areas some studies describe a second, rostrally located forelimb area of representation separated by head, neck, and vibrissae representations (Gioanni et al., 1985; Neafsey et al., 1986).

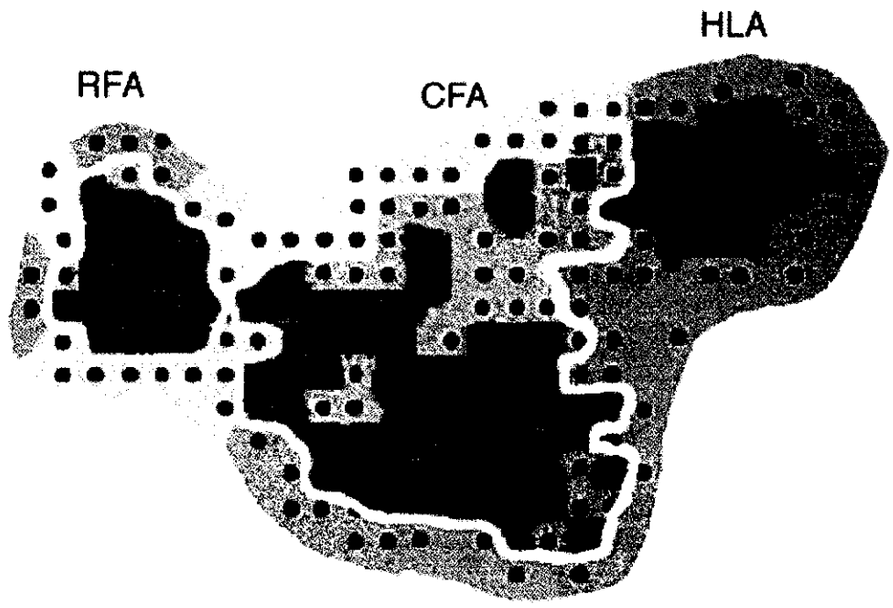

Figure 1.5 - Topographic representations within the rat motor cortex. Rostral forelimb area (RFA), caudal forelimb area (CFA), and hindlimb area (HLA) consist of digit (red), wrist (green), elbow (light blue), and hindlimb (dark blue) movements. These areas are bordered by head/neck/vibrissae (yellow) representations and non-responsive sites (grey).

The general layout of somatotopic subdivisions is consistent across animals but the location of the two forelimb areas in relation to the overlap of the motor and somatosensory cortex differs. The rostral forelimb area (RFA) is the sole group of forelimb representations located within the agranular cortex, which characterizes motor cortex (Neafsey and Sievert, 1982). The caudal forelimb area (CFA) overlaps with somatosensory cortex or granular cortex which is also activated by sensory stimuli (Donoghue, 1985; Neafsey et al., 1986; Gu et al., 1999). The internal organization of these areas differs between animals. Within the CFA and the RFA there are several noncontinguous representations of the same joint (Weiss and Keller, 1994). Some

authors propose that certain forelimb movements are evoked when specific sites of the RFA and CFA are stimulated (Neafsey et al., 1986). Other investigations have reported each muscle does not have its own representation in a corresponding limb area but the muscle can be excited from points in almost the whole area (Gioanni et al., 1985). Many investigators propose that representations are organized into small clusters of corticomotoneuronal cells which facilitate a common group of muscles (Asanuma et al., 1972; Kwan et al., 1978; Neafsey, 1980; Cheney and Fetz, 1985). In other words, neighboring pyramidal cells influence the same group of muscles.

The topography of movement representations within rat primary motor cortex has been shown to change following acquisition of a motor skill. The paradigm used in the work presented in this thesis requires a rat to learn a novel motor skill of reaching for a food pellet through a narrow slot. Rats are not normally required to perform this task to obtain food. Following acquisition of this motor skill, ICMS techniques reveal there is a shift from equal proximal and distal forelimb representations within the CFA to predominant distal representations (Kleim et al., 1998). When ischemia is induced within a movement representation, there are subsequent behavioral deficits (Nudo et al., 1996b; Nudo et al., 2000b). Following further training, however, there is improvement in performance (Goertzen et al., 2001). Therefore, ICMS provides the window to see functional organizational changes following learning, ischemia, and behavioral recovery.

The rat's highly developed motor system, including the large repertoire of behaviors, provides the ideal model to study the relationship between cortical organization of motor behaviors and the role of the cortex in controlling movement. Intrinsic cortical connections provide the foundation for rat motor cortex somatotopic

movement representations and intracortical microstimulation provides the method by which to study changes in representation following manipulations such as skill learning or ischemic damage or a combination of both. Since topography of movement representations are altered following acquisition of a motor skill (Kleim et al., 1998; Remple et al., 2001), an ischemic event (VandenBerg and Kleim, 2001), and rehabilitative recovery (Goertzen et al., 2001), this model provides the framework to uncover the idiosyncrasies in the relationship between peri-infarct diaschisis, ischemia-induced behavioral deficits, and behavioral recovery following rehabilitation.

VI. OUTLINE OF THESIS

The goal of this thesis is to examine the relationship between post-ischemic diaschisis and rehabilitation-dependent behavioral improvement. To this end, I will take advantage of a rodent model of ischemia involving rat forelimb motor cortex and a skilled reaching behavior. Intracortical microstimulation will provide the method of measuring diaschisis evaluating in the motor cortex following stroke. The reaching task will provide the manner in which to measure behavioral deficits and administer a rehabilitation regimen. The recovery of forelimb use following the induction of ischemia seems to be supported by functional compensation within the forelimb motor cortex and seems to be dependent on behavioral rehabilitation.

Two primary questions will be addressed: The first question is how soon after ischemic insult does diaschisis appear? Second, is there a critical window during which rehabilitation must be given in order to achieve behavioral recovery and overcome diaschisis?

CHAPTER 2:

ASSOCIATIVE DIASCHISIS WITHIN MOTOR CORTEX OCCURS 24 HOURS AFTER ISCHEMIC INSULT

INTRODUCTION

In the early 1900s, von Monakow introduced the term "diaschisis" to explain behavioral symptoms in clinical cases that could not be accounted for by the size of the lesion. He described diaschisis as a sudden interruption of function originating from a focal lesion that reached areas of the brain outside the locus of damage (von Monakow, 1914). He proposed three types of diaschisis including functional depression from primary motor cortex to the spinal cord (cortico-spinalis), dysfunction within the contralateral cortex (commissuralis), and intracortical fiber-mediated dysfunction in intact cortical areas neighboring the locus of injury (associative; Feeney et al., 1986). Recent research has provided examples of associative diaschisis. In the lesioned hemisphere hyperexcitability has been reported (Domann et al., 1993; Buchkremer-Ratzmann et al., 1998). GABA (γ-aminobyturic acid) mediated synaptic inhibition is also less efficient adjacent to lesioned areas (Neumann-Haefelin et al., 1995) possibly due to a decrease in $GABA_A$ receptor expression ipsilateral to the lesion (Schiene et al., 1996).

Although the relationship between diaschisis and behavioral impairment is unclear, there is some evidence that it may contribute to motor dysfunction. Traversa et al., (1997) have shown using focal transcranial magnetic stimulation (TMS), that there is a significant increase in excitatory thresholds and latency of motor evoked potentials, and a significant decrease in motor output area in the affected hemisphere following stroke. Nudo and Milliken (1996a) have shown that following an infarct there was a transient decrease in performance of skilled digit use associated with a decrease in the area of digit representations and a simultaneous increase in proximal representations. Similar effects have been shown in the rat motor cortex in which there was a decrease in skilled forelimb

use following ischemia associated with a loss of forelimb movement representations extending beyond the infarction (Goertzen et al., 2001).

The alleviation of associative diaschisis may also contribute to behavioral improvements observed following trauma. For example, using functional magnetic resonance imaging (fMRI), it has been demonstrated that following a stroke, patients exhibit a larger volume of activation associated with increased recovery (Cramer et al., 2001). Also, patients present peri-infarct activation that is hypothesized to reflect cortical map reorganization, reliance on alternative motor sites, or both (Cramer et al., 1997; Cramer et al., 2000). In monkey studies, it has been shown that if there is a lesion in the motor representation of the hand, these representations reappeared in the zone surrounding the infarct (Glees and Cole, 1950). Animal studies have shown deficits in skilled forelimb performance associated with a loss of forelimb movement representations within peri-infarct cortex. Both the impairments and loss of movement representations can be reversed with the administration of skilled rehabilitative training (Friel et al., 2000; Goertzen et al., 2001).

All of these studies have examined peri-infarct dysfunction days to weeks after insult. How quickly this dysfunction develops post-insult is unknown. Understanding the time course of dysfunction may provide insight into cellular mechanisms underlying diaschisis and associated behavioral impairments. In this study, we investigated the etiology of diaschisis-induced dysfunction in movement responses of the rat caudal forelimb area at one and twenty-four hours following focal ischemia. Specifically, we evaluated the presence of diaschisis within peri-infarct cortex one and twenty-four hours following ischemia. Examination at one hour following insult evaluated immediate

changes in movement representations. Previous studies evaluate changes after rehabilitation is administered. If diaschisis contributes to motor impairments, then it should be present prior to rehabilitation.

MATERIALS AND METHOD

Animals:

Thirty-one male Long-Evans hooded rats approximately four months of age (350-550g) were randomly assigned to either a One Hour (1Hr) Condition (n=14) or a Twenty-Four (24Hr) Condition (n=17), with littermates equally distributed across conditions. Following the first intracortical microstimulation (ICMS) session, animals in each condition were then randomly assigned to either the Control (n=16) or the Ischemic (n=15) Conditions. Control animals did not receive an electrocoagulation lesion while the Ischemic Condition did receive a lesion immediately following the first electrophysiological mapping session. The 1Hr Condition were allowed to sit one hour between ICMS mapping sessions and the 24Hr Condition were allowed to recover for twenty-four hours before mapping was repeated.

Electrophysiological Mapping:

Standard intracortical microstimulation (ICMS) techniques were used to generate detailed maps of forelimb representations within the motor cortex (Kleim et al., 2002). All animals were food deprived for sixteen hours prior to each ICMS session. Prior to surgery animals were anaesthetized with ketamine hydrochloride (70 mg/kg i.p.) and xylazine (5 mg/kg i.m.), receiving acepromazine (0.02 mg/kg i.p.) and ketamine (20 mg/kg i.m.) as needed. A craniotomy was performed randomly over either the left or right motor cortex. A small puncture was made in the cisterna magna to reduce edema

prior to retraction of the dura. The exposed cortex was then covered with warm silicon oil (37°C). A glass microelectrode controlled by a hydraulic microdrive was used to make penetrations to a depth of ~1550 μm (corresponding to cortical layer V) with an interpenetration distance of 375 μm. Stimulation consisted of thirteen, 200 μs cathodal pulses delivered at 350 Hz from an electrically isolated stimulation circuit. Animals were maintained in a prone position, with consistent limb support. At each penetration site, the minimal threshold required to elicit a movement was recorded and sites where no movement was detected at ≤ 60 μA were recorded as non-responsive. 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 areal extent of the caudal forelimb area (CFA) (Remple et al., 2001). The mean stimulation threshold (μA) for each movement category (distal/proximal) was also calculated (Kleim et al., 2002).

Cortical Infarction:

Immediately following mapping, Ischemic animals received a cortical infarct within approximately 30% of the distal movement representations contained within the CFA. The infarct was created via electrocoagulation of all surface vasculature within the targeted area until vasculature was no longer visible (Nudo et al., 1996a). The infarcted vessels included very thin capillaries as well as larger vessels. All bypassing vasculature was avoided in order to confirm the damage to the specific physiologically defined area. Potential reperfusion was examined for two minutes. If reperfusion was observed, the tissue was again coagulated until the vasculature was no longer visible. Peri-infarct cortex was defined as the CFA representations outside the region in which surface

vasculature was coagulated. The 1Hr Condition remained in the stereotaxic apparatus under anesthesia between mapping sessions. In the 24Hr Condition, the craniotomy was cleared of silicon oil and closed with gel film and gel foam. SDI Wave flowable composite dental epoxy was applied to the opening and then cured with Dentsply QHI75 UV Light until hardened. The cisterna magna incision was sealed and the scalp was closed with wound clips. Body temperature was monitored as the animals were allowed to recover for twenty-four hours in a single animal housing unit and administered warm Ringers Solution (4 cc/hr s.c.) until awake.

RESULTS

CFA Representation

A Student's t-test (two-tailed, dependent, $p<0.05$) revealed a significant expansion of CFA area (mm^2) in Control ($t(6)=-2.88$, $p=0.028$, Figure 2.1A; 2.2A) but not Ischemic animals ($t(6)=0.12$, $p=0.91$, Figure 2.1B; 2.2B) after one hour. No significant difference in area of distal ($t(6)=1.53$, $p=0.17$) or proximal ($t(6)=-1.31$, $p=0.25$) movement representations between Map 1 and 2 in 1Hr Ischemic animals was found. A significant increase was found in the 1Hr Control Condition for distal ($t(6)=-4.91$, $p=0.003$) but not proximal area ($t(6)=0.94$, $p=0.32$) between Maps 1 and 2 (Table 2.1). A Student's t-test (two-tailed, dependent, $p<0.05$) revealed a significant decrease in the total area of peri-infarct representations in the Ischemic Condition ($t(7)=3.75$, $p=0.01$, Figure 2.1D) but not Control animals ($t(8)=-1.84$, $p=0.10$, Figure 2.1C) after twenty-four hours. There was no significant difference in area of distal ($t(6)=1.70$, $p=0.13$) or proximal ($t(7)=1.11$, $p=0.31$) movement representations in the 24Hr Ischemic condition. In the 24Hr Control

condition, there was no significant difference in area of distal ($t(8)=-2.17$, $p=0.06$) and proximal ($t(8)=-0.61$, $p=0.56$) movement representations within CFA (Table 2.2).

Table 2.1 - 1 Hr Condition Distal and Proximal Representation Area (mm^2).
Mean area (mm^2, +/- SEM) of distal and proximal forelimb representations for Control and Lesion 1 Hr. Conditions. A significant increase in distal representations was found in the Control Condition (*Student's t-test, $p<0.05$).

	CONTROL		ISCHEMIC	
	Map 1	**Map 2**	**Map 1**	**Map 2**
Distal	3.1 ± 0.6	4.1* ± 0.5	2.4 ± 0.2	2.1 ± 0.3
Proximal	1.9 ± 0.3	2.3 ± 0.3	1.2 ± 0.2	1.6 ± 0.3

Table 2.2 - 24 Hr Condition Distal and Proximal Representation Area (mm^2).
Mean area (mm^2, +/- SEM) of distal and proximal forelimb representations for Control and Lesion 24 Hr. Conditions. No significant differences were found for distal and proximal representations between Control and Lesion Conditions.

	CONTROL		ISCHEMIC	
	Map 1	**Map 2**	**Map 1**	**Map 2**
Distal	2.2 ± 0.3	2.7 ± 0.3	1.7 ± 0.2	1.2 ± 0.2
Proximal	1.5 ± 0.2	1.6 ± 0.2	1.1 ± 0.3	0.6 ± 0.2

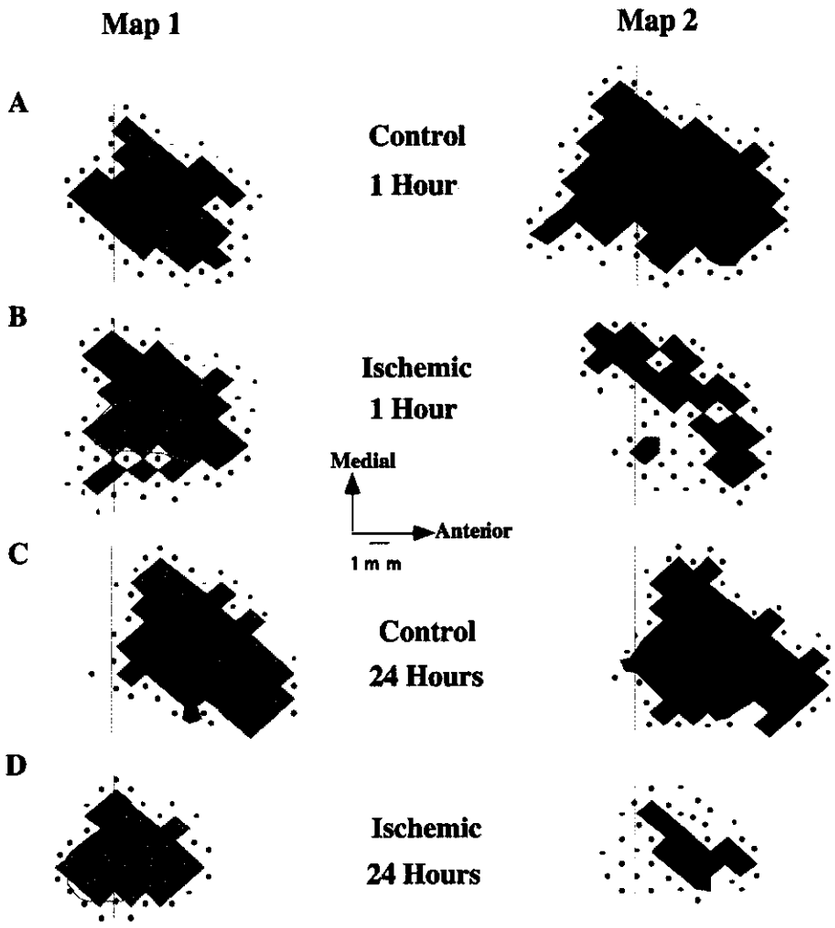

Figure 2.1 - Representative motor maps from 1 Hr Control (A), 1 Hr Ischemic (B), 24 Hr Control (C), and 24 Hr Ischemic Conditions derived using ICMS. 1 Hr Control animals exhibited a significant increase in distal (green) but not proximal (blue) representations. 24 Hr Ischemic animals showed a significant decrease in peri-infarct movement representations in Map 2. The red line depicts Bregma and the red circle represents the area of ischemic insult.

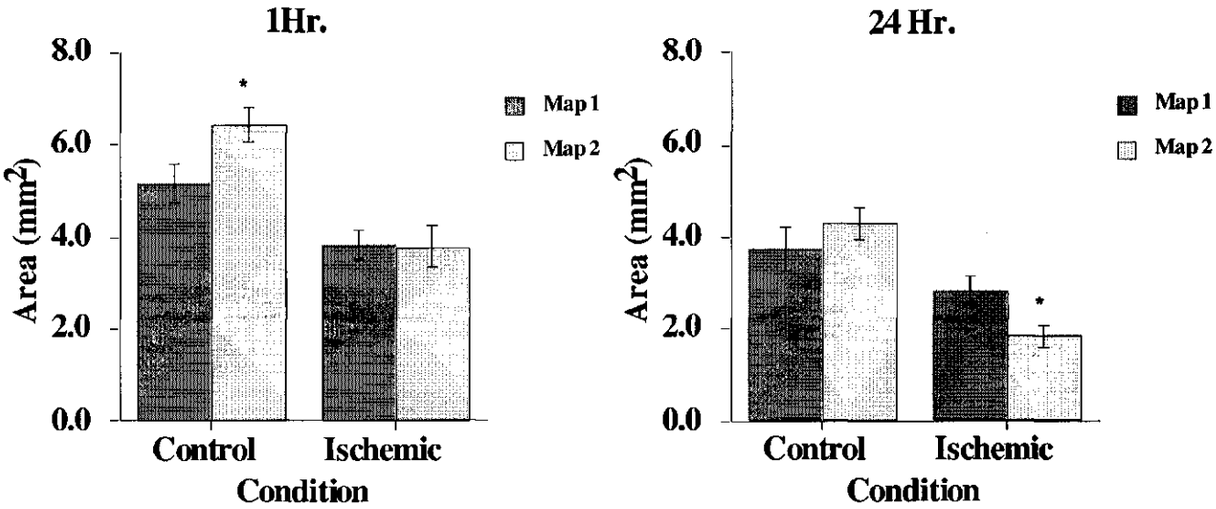

Figure 2.2 - Total area of CFA (mm^2) after 1 Hr (A) and 24 Hr (B). There is a significant increase in CFA area one hour following ICMS and a significant decrease in peri-infarct representation twenty-four hours following ischemia (*$p<0.05$; two-tailed, dependent Student's t-test).

Movement Thresholds

A Student's t-test (two-tailed, dependent, $p<0.05$) showed no significant differences in the 1Hr Ischemic Condition for distal ($t(6)=-1.57$, $p=0.17$) or proximal ($t(6)=-0.56$, $p=0.60$) thresholds between Maps 1 and 2 (Table 2.3). There was no significant difference in the distal ($t(6)=0.49$, $p=0.64$) or proximal ($t(6)=0.78$, $p=0.46$) thresholds for 1Hr Control animals. A significant decrease in distal movement thresholds ($t(7)=-3.77$, $p=0.01$) was found without significant differences in proximal movement thresholds ($t(7)=-1.33$, $p=0.23$) from Map 1 to 2 in the 24Hr Ischemic Condition. No significant difference was found in the thresholds of distal ($t(8)=0.02$, $p=0.99$) and proximal ($t(8)=1.30$, $p=0.23$) movements in the 24Hr Control Condition (Table 2.4).

Table 2.3 - 1Hr Condition Thresholds (μA).

Mean threshold values for Control and Lesion 1 Hr Conditions (+/- SEM). No significant differences were found between Lesion and Control Animals after one hour (+/- SEM).

	CONTROL			ISCHEMIC		
	Proximal	**Distal**	**Average**	**Proximal**	**Distal**	**Average**
MAP 1	27.5 ± 1.6	26.2 ± 1.9	26.9 ± 1.4	27.3 ± 1.1	25.8 ± 1.3	26.3 ± 1.0
MAP 2	24.9 ± 2.3	24.9 ± 1.6	25.2 ± 1.9	28.4 ± 1.5	27.9 ± 0.5	28.2 ± 0.8

Table 2.4 - 24Hr Condition Thresholds (μA).

Mean threshold values for Control and Lesion 24Hr Conditions (+/- SEM). A significant increase in distal forelimb movement thresholds from Map 1 to 2 was observed in the Lesion Condition (*Student's t-test, $p<0.05$).

	CONTROL			ISCHEMIC		
	Proximal	**Distal**	**Average**	**Proximal**	**Distal**	**Average**
MAP 1	32.4 ± 3.1	28.4 ± 3.0	30.5 ± 1.7	30.1 ± 1.7	15.1* ± 6.2	32.7 ± 0.8
MAP 2	28.8 ± 2.0	28.4 ± 2.0	29.3 ± 1.8	33.7 ± 2.5	33.7* ± 2.0	33.7 ± 1.9

DISCUSSION

There have been numerous demonstrations of the capacity for reorganization within motor maps in response to a variety of manipulations (Sanes et al., 2000). For example, changes in motor map topography can result following learning (Kleim et al., 1998), amputation (Donoghue et al., 1987), or changes in tactile information (Keller et al., 1996). Here we show the topographic representation of forelimb movements within

the rat motor cortex is dramatically influenced by standard ICMS techniques and also by focal ischemia. ICMS alone is sufficient to cause a significant expansion of the CFA one hour after the first mapping session, which subsided by twenty-four hours. However, an ischemic event seems to prevent the ICMS-induced expansion of the CFA at one hour. Finally, associative diaschisis represented by the absence of evoked movement responses in intact areas of the motor cortex surrounding the ischemic tissue was found at twenty-four hours accompanied by a decrease in threshold needed to elicit distal movement responses.

The finding that ICMS can alter the pattern of movement representations within the motor cortex is not new. Early research performed by Sherrington and his colleagues (1912) reported the instability of cortical points in which the border of motor representations are not sharp or abrupt edges, and facilitation seems to make the border extend further than it would in the absence of facilitation. Facilitation of representations via ICMS has been shown to activate pyramidal cells directly and trans-synaptically across the motor cortex (Stoney et al., 1968). It has also been found that indirect polysynaptic activation of pyramidal tract cells in the monkey and cat motor cortex significantly increased when repetitive intracortical stimuli was applied (Jankowska et al., 1975). Recent research has shown that following repetitive ICMS in rats there is an expansion of forelimb representational borders of at least 200 μm (Nudo et al., 1990). Similar results were found in the receptive fields of the auditory cortex of rats. When the cortex was stimulated using ICMS, there was an increase in the cortical representation of a particular acoustic frequency that had been represented at the site of the stimulating electrode (Maldonado and Gerstein, 1996).

The expansion of movement representations following repetitive ICMS may be due to the structure of the cortex. It has been reported that there are long reaching pyramidal cell collaterals within the motor cortex (Asanuma et al., 1976) and that there is widespread cortical activation following an ICMS pulse (Baker et al., 1998). Excitation or disinhibition of pyramidal cell collaterals within the motor cortex may promote the expansion of movement representations. Following repetitive transcranial magnetic stimulation (rTMS) in humans there is an overall increase in the excitability of the cortex in association with an increase in the size of muscle-evoked potentials (MEPs) from the first to the last stimulation (Berardelli et al., 1998). With repeated seizure activity in the form of kindling there is an increase in synaptic strength accompanied by a doubling in the area of the rat forelimb representations (Teskey et al., 2001). Following pharmacological blockade of the inhibition of the motor cortex, movements of neighboring representations were evoked by stimulation in adjacent areas (Jacobs and Donoghue, 1991). These authors propose that alterations of inhibitory connections shape movement representations. Therefore, repetitive use of standard ICMS techniques increases the size of movement representations within the CFA of the rat motor cortex possibly by altering the excitability or strength of inhibition of pyramidal cells or the efficacy of synapses within Layer V.

Despite the increased map size observed with ICMS in 1Hr Control animals, no such expansion is observed in animals with a focal ischemic event reflecting an alteration in the excitability of the cortex. At twenty-four hours the changes in excitability may still be present. In 24Hr Lesion animals there is a decrease in threshold necessary to elicit forelimb movement accompanied by a decrease in forelimb representation. A decrease in

threshold reflects an increase in excitability. However, a decrease in motor map size suggests a decrease in excitability of the cortex. It is possible that both mechanisms are happening simultaneously. Lower thresholds may be the result of a massive extracellular accumulation of GABA post-ischemia (Phillis et al., 1994) preventing facilitation in 1Hr Lesion animals. This massive release causes a disruption of the GABA system via decreased GABA synthesis (Schwartz-Bloom and Sah, 2001), a downregulation of $GABA_A$ receptors (Schiene et al., 1996), and decreases in $GABA_A$ receptor mRNA (Li et al., 1993). On the other hand, decreases in map size may be facilitated by the insensitivity of interneurons. Interneurons are less sensitive to ischemia (Johansen et al., 1983; Nitsch et al., 1989) and stimulation post-ischemia may be preferentially driving interneurons in some areas, therefore decreasing map size.

Regardless of the mechanism, cortical dysfunction manifestations begin within one hour after insult and a loss of movement representations occurs by twenty-four hours. Our results confirm previous findings that there is a loss of motor representations within ischemic cortex (Nudo et al., 1996a, b; Friel et al., 1998; Goertzen et al., 2001) at one and twenty-four hours post-ischemia accompanied by an abnormal absence of motor representations within intact areas of the motor cortex. The diaschitic loss of movement representations has been a suggested cause of behavioral impairments (Nudo et al., 1996a, b; Friel et al., 1998). Following an ischemic infarct there are initial transient motor deficits followed by spontaneous recovery in the absence of rehabilitation associated with a permanent loss of movement representations (Nudo et al., 1996a). However, if rehabilitation is administered following an ischemic event movement representations are retained (Friel et al., 1998) in association with an increase in

behavioral skill (Nudo et al., 1996b; Goertzen et al., 2001). It is possible that the resolution of the initial loss of movement representations in intact areas seen at twenty-four hours is responsible for spontaneous behavioral recovery and facilitates the retention of representations when rehabilitation is administered following ischemia.

The present experiment indicates that not only does ischemia influence the topographical representation of movement within the motor cortex but standard ICMS can as well. Repetitive stimulation induced by ICMS seems to cause a change in the responsiveness of the cortex reflected in increases in the size of movement representations. The effects of a permanent focal ischemic event counteract this dramatic expansion. Ischemia also induces associative diaschisis depicted in the absence of movement representations in intact but dysfunctional areas of the motor cortex. It has been previously hypothesized that perifocal tissue has a transient suppression of function that recovers its original excitability during the course of recovery (Glassman, 1971). Alleviation of this transient phenomenon may be the first step towards behavioral recovery. The occurrence of peri-lesional dysfunction at twenty-four hours indicates a need for early therapeutic interventions following stroke to alleviate peri-infarct diaschisis.

CHAPTER 3:

EVALUATION OF THE EFFECTS OF DELAYED REHABILITATION ON BEHAVIORAL RECOVERY AND FUNCTIONAL ORGANIZATION OF THE RAT MOTOR CORTEX.

INTRODUCTION

Almost a century ago, von Monakow introduced the term "diaschisis" to explain how focal brain lesions lead to dysfunction both distal and proximal to the insult (von Monakow, 1914). Recently, modern neurophysiological techniques have provided support for this hypothesis. Positron emission tomography (PET; Weiller et al., 1993), functional magnetic resonance (fMRI; Cramer et al., 1997), and intracortical microstimulation (ICMS; Nudo et al., 1996a, b) studies have all shown loss of function within the cortex following ischemia. Furthermore, it has been shown that the alleviation of diaschisis is accompanied by the resolution of behavioral deficits. For example, after a focal infarct within the hand representation of squirrel monkeys, animals recovered the skilled use of forelimb even in the absence of rehabilitation to near pre-lesion levels. The recovery of forelimb use was associated with a shift from distal to proximal extremity representations (Nudo et al., 1996a). Recovery associated with organizational shifts suggests that functional restoration may be the result of the alleviation of peri-lesional dysfunction or diaschisis.

Other investigations support the idea that rehabilitation enhances functional and behavioral recovery and further alleviates diaschitic dysfunction (Goertzen et al., 2001). In human studies using constraint-induced therapy of the impaired limb, there is an alleviation of diaschisis in motor cortex associated with long-term improvement of use of the impaired limb (Taub et al., 1993). To further enhance recovery and also retain function within the motor cortex it has been shown that repetitive use is more beneficial than a lack of rehabilitation (Friel et al., 2000). However, it seems that skilled training may be more advantageous. When rats are trained in skilled and unskilled rehabilitation

paradigms, the rats in the skilled reach training condition not only improve in behavioral performance but also retain a greater amount of peri-lesional representations similar to those damaged by ischemia in adjacent cortex (Goertzen et al., 2001). This study indicates the need for skilled movement as a rehabilitative therapy.

Although the ability of rehabilitation to improve function is clear, the amount of recovery may depend on the type of rehabilitation. For example, if the ipsilateral forelimb of a rat is casted immediately following ischemia and the animal is forced to use the impaired limb, exaggerated sensorimotor deficits emerge (Bland et al., 2000). In rats, if animals are forced to use the impaired limb for the first fifteen days following a lesion there are severe and long lasting behavioral deficits and blocked use-dependent dendritic growth of layer V pyramidal neurons seen in other studies (Kozlowski et al., 1996). Forced overuse of the impaired limb in the first seven days caused a greater expansion of lesion size and interfered with the restoration of function more than forced use during the second seven days (Humm et al., 1999). However, in the absence of forced use as an intervention authors report functional and behavioral improvements (van der Lee et al., 1999).

Not only does recovery depend on the type of rehabilitation administered but it also depends on the timing of rehabilitation administration. Five days following ischemia, monkeys exhibit a period of rapid improvement in manual skill, however, they experience a relapse of skill levels apparent immediately after infarct. This relapse in performance is followed by a second period of improvement and finally stabilization to normal levels (Nudo et al., 1996b). When post-lesional training of the weak limb is delayed for four months in the rhesus monkey, recovery of strength in the impaired limb

is less effective than when rehabilitation is started immediately after stroke (Black et al., 1975). The data suggest that the effectiveness of rehabilitation may be dependent on the time of intervention after insult.

The present experiment was designed to determine whether skilled rehabilitation would facilitate behavioral recovery and alleviate peri-infarct diaschisis following a delay. Also, we investigated whether the level of functional recovery was dependent on the timing of rehabilitation onset. Finally, we examined whether skilled rehabilitation itself was necessary to facilitate behavioral recovery and alleviate diaschisis following ischemia.

MATERIALS AND METHOD

Animals:

Forty-five male Long Evans hooded rats (Canadian Centre for Behavioural Neuroscience colony) approximately three months of age (365-560g) were housed in home cages (11x40x40 cm) on a 12:12 light/dark cycle. All animals were placed on a restricted diet (15g/day/animal) until body weight was reduced to 90% of their adult body weight. Animals were randomly assigned to the Rehabilitation (RH, n=32) or Non-rehabilitation (NRH, n=12) Conditions (Fig. 1). Animals in the RH Condition were assigned to either a Control (n=17) or Ischemic Condition (n=15) with littermates equally distributed across conditions. An intracortical microstimulation (ICMS) session was performed on the forelimb area of each animal, which were then assigned to either the fourteen-day (14-RH; n=16) or twenty-eight-day (28-RH; n=16) Rehabilitation Condition. Control animals did not receive an electrocoagulation lesion whereas the Ischemic Condition did immediately following the first electrophysiological mapping

session. The 14-RH Condition were allowed to recover for fourteen days and 28-RH animals were allowed to sit for twenty-eight days in their home cages before ten days of rehabilitative training was administered. In the Non-Rehabilitation Condition (NRH; n=12) all animals were assigned to the Ischemic Condition and remained in their home cages for fourteen days (14-NRH) or twenty-eight days (28-NRH) following the first ICMS session and focal ischemic infarct. NRH animals sat an additional ten days instead of rehabilitative training before a probe trial was administered. After training and probe trials all the animals were remapped.

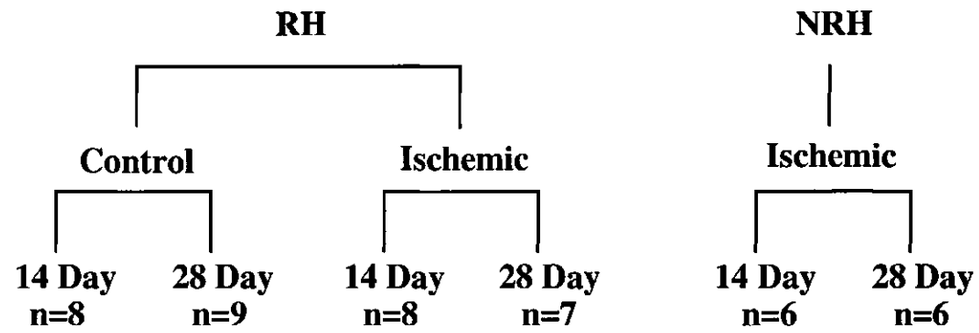

Figure 3.1: Experimental design for fourteen day (14-RH) delay Control and Ischemic Conditions, for twenty-eight day (28-RH) delay Control and Ischemic Conditions, and for Non-rehabilitation (NRH) Conditions.

Skilled Reach Training:

Before receiving the first session of ICMS, all animals were trained on a skilled reaching paradigm (Whishaw and Miklyaeva, 1996). Prior to single pellet reach training, animals were subjected to three days of pretraining in order to familiarize the animals with the reaching paradigm. The pretraining box consisted of a 20 x 28 x 26 cm Plexiglas cage with a wire mesh bottom that did not allow animals to retrieve dropped food pellets (45mg, Rodent Chow food pellets, Bioserve Inc, PO Box 250 Frenchtown,

NJ). The cage had a 5.4 x 20 x 0.5 cm tray for holding pellets directly outside of the vertical bars at the front of the cage. The rats could reach through the bars of the cage with one or both paws to grasp pellets and had to maintain their grip on the pellets in order to transfer them to their mouth. On the first day, the animals were encouraged to reach through the bars to retrieve pellets. The subsequent days provided opportunity to establish limb preference and for the animals to practice pellet retrieval.

On the fourth day, following acquisition of the reaching paradigm all animals were trained on a skilled single pellet reaching task. Animals were placed into a Plexiglas box (11.5 x 40 x 40cm) with a 1cm wide slit in the front panel. Under the slot was a platform (3 x 10cm) that contained two shallow wells (0.25cm deep) in which a food pellet could be placed. The animal was required to reach through the slot to grasp the pellet. A successful reach was defined as one in which the animal approached the slot, reached to the well, retrieved the pellet in a single reach or grasping motion, and transferred the pellet to his mouth without dropping it. The animal was trained for ten minutes per day, for a minimum of ten days until approximately 50% accuracy criterion was reached. Each training session was recorded using a Sony Digital Video Camera Recorder (Model number DCR-PC9) onto Memorex SHQ T-120 VHS tapes. Preferred paw was recorded for each animal. Accuracy was measured by dividing the number of pellets retrieved by the number of total reaches within the last training session.

Electrophysiological Mapping:

Standard intracortical microstimulation (ICMS) techniques were used to generate detailed maps of forelimb representations within the rat motor cortex (Kleim et al., 2002). All animals were food deprived for sixteen hours prior to each ICMS session. Animals

were anaesthetized with ketamine hydrochloride (70 mg/kg i.p.) and xylazine (5 mg/kg i.m.), receiving acepromazine (0.02 mg/kg i.p.) and ketamine (20 mg/kg i.p.) as needed. A craniotomy was performed over the motor cortex in the hemisphere contralateral to the preferred paw used in the reaching task. A small puncture was made in the cisterna magna to reduce edema prior to retraction of the dura. The exposed cortex was then covered with warm silicon oil (37°C). A glass microelectrode controlled by a hydraulic microdrive was used to make penetrations to a depth of ~1550 μm (corresponding to cortical layer V) with an interpenetration distance of 375 μm. Stimulation consisted of thirteen, 200 μs cathodal pulses delivered at 350 Hz from an electrically isolated stimulation circuit. Animals were maintained in a prone position with consistent limb support. At each penetration site, the minimal threshold required to elicit a movement was recorded and sites where no movement was detected at ≤ 60μA were recorded as non-responsive. Breathing rate was monitored to assess anesthetic level and temperature was maintained at 37°C. 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 areal extent of caudal forelimb area (CFA; Remple et al., 2001) defined as the forelimb representations caudal to and bordered by head/neck and vibrissae representations and rostral to and bordered by hindlimb and trunk representations.

Cortical Infarction:

Immediately following the initial mapping session, a cortical infarct was made within approximately 30% of the distal movement representations contained within the CFA. The infarct was created via electrocoagulation of all the surface vasculature within

the targeted area (Figure 3.2; 3.3; Nudo et al., 1996a). The craniotomy was cleared of silicon oil and closed with gel film and gel foam. SDI Wave flowable composite dental epoxy was applied to the opening and then cured with Dentsply QH175 UV Light for approximately fifty seconds. The cisterna magna incision was sealed and the scalp was sutured closed. Body temperature was monitored as the animals were allowed to recover for twenty-four hours in a single animals housing unit and administered warm Ringers solution (4 cc/hr s.c.) until awake.

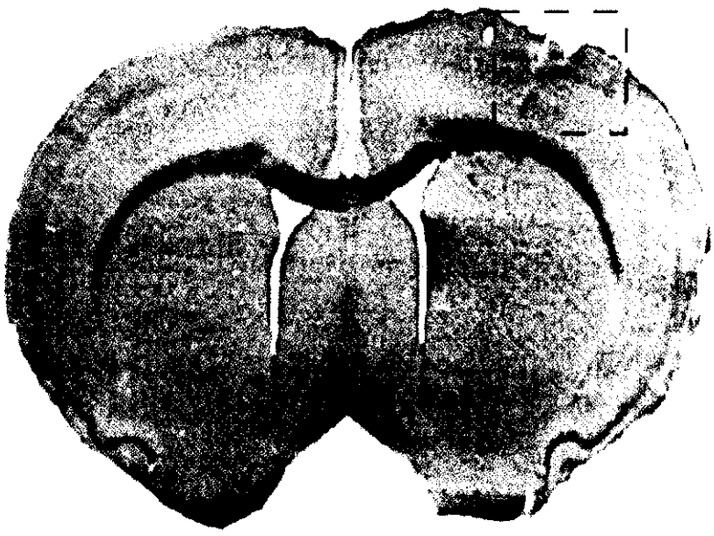

Figure 3.2 - Section of rat brain tissue (40 μm) approximately 0.95 μm anterior to Bregma corresponding to primary motor cortex. The area within the box represents area of cortical infarction.

Figure 3.3 - Magnified section of rat brain tissue (40 μm; 4 x) from box area in Figure 3.2. Red line surrounds area of damage resulting from cortical infarction via bipolar coagulation of surface vasculature.

Rehabilitative Training:

Rehabilitation training was administered in the form of the single pellet reaching task that was administered prior to the first ICMS session and the ischemic event. After a fourteen-day (14-RH) or twenty-eight-day delay (28-RH) animals received ten minutes of skilled single pellet reach training per day for ten days. The task was administered in the same manner as the skilled training task. During this ten day period, the NRH Condition remained in their home cage. Before the second mapping session, a probe trial was administered to the NRH animals to assess reaching ability. The probe trial consisted of a single pellet reaching session in which the percent success for twenty reach attempts

was recorded. Evaluation of performance in rehabilitation was compared to pre-lesion scores by combining days one and two (early phase), days five and six (middle phase), and days nine and ten (late phase).

RESULTS

Behavioral Performance

i. Accuracy

Following pretraining on the reaching task, all animals could successfully retrieve pellets from the tray. Paw preference was also evident. When trained on the single pellet reaching task, animals reached criteria by scoring fifty percent or higher on reaching accuracy scores after day ten of training.

A repeated measures ANOVA with DAY as a within subject factor and TIME and CONDITION revealed a significant DAY x CONDITION interaction ($F(3,84)=2.90$; $p<0.05$; Fig 1). There was no significant effect of TIME (14 and 28 day) so these data were collapsed into either Ischemic or Control Conditions (Figure 3.4). Subsequent multiple comparisons (Fischer's PLSD, $p<0.05$) showed Ischemic RH animals to have significantly lower reaching accuracy than Control RH animals at the early time point during rehabilitative training. Further, NRH animals had significantly reduced reaching accuracy than both Ischemic and Control RH animals at the late time point (Figure 3.5).

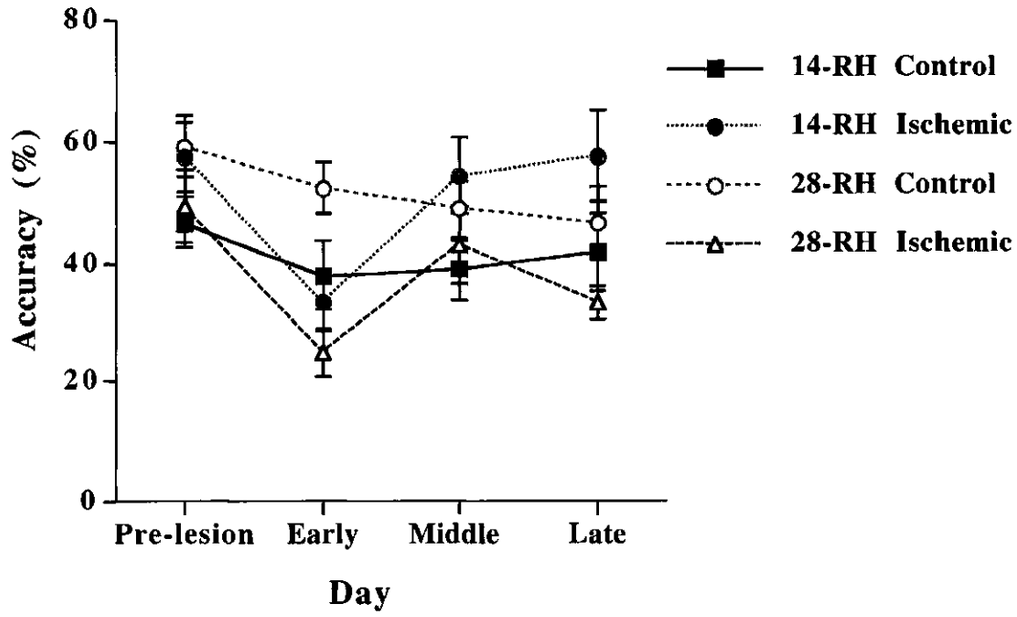

Figure 3.4 - Mean reaching accuracy (%) for fourteen-day (14-RH) Control and Ischemic Conditions and for twenty-eight-day (28-RH) Control and Ischemic Conditions on Prelesion, Early (days 1 and 2), Middle (days 5 and 6), and Late (days 9 and 10) time points. There was no significant effect of TIME ($p<0.05$).

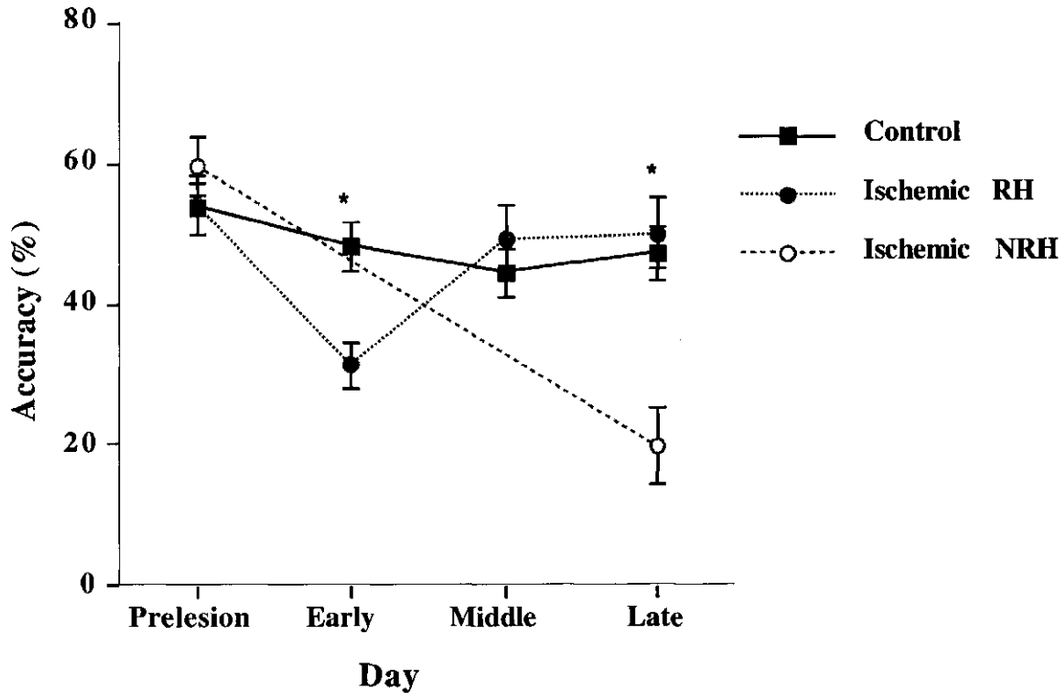

Figure 3.5 - Mean reaching accuracy (%) for Control, Ischemic Rehabilitation (RH), and Ischemic Non-rehabilitation (NRH) Conditions on Prelesion, Early (days 1 and 2), Middle (days 5 and 6), and Late (days 9 and 10) time points. Controls and Lesion RH animals performed significantly better than NRH animals by the last day of rehabilitation (*$p<0.05$).

Area of CFA

Animals in each condition were excluded from statistical analysis if the second map was not attained because of death before second surgery or because the map data was more than two standard deviations from the mean (Table 3.1). A repeated measures Analysis of Variance (ANOVA) with TREATMENT and TIME as between subject factors and MAP TIME as within subject factor revealed a significant effect of CONDITION ($F(1,39)=10.1$, $p<0.05$) and MAP TIME ($F(1,39)=94.8$, $p<0.001$)on map area between Map 1 and Map 2 (Figure 7A; 8). Because there were no significant differences between either the 14-RH and 28-RH Conditions (Figure 3.6) in either Ischemic or Control groups, these groups were collapsed. A further ANOVA with

CONDITION as a between subject factor revealed a significant effect of CONDITION on the percentage of peri-infarct tissue in Map 2 ($F(2,40)=3.48$, $p<0.05$). Subsequent multiple comparisons (Fisher's PLSD, $p<0.05$) showed NRH animals to have significantly lower percentage of peri-infarct movement representations than both RH Ischemic and RH Control animals (Figure 3.7B).

Table 3.1: Animals included in statistical analysis of Area of CFA for fourteen day (14-RH) delay Control and Ischemic Conditions, for twenty-eight day delay (28-RH) Control and Ischemic Conditions, and for Non-rehabilitation (NRH) Conditions.

	RH		NRH
	Control	**Ischemic**	**Ischemic**
14 - Day	8	8	5
28 - Day	7	8	6

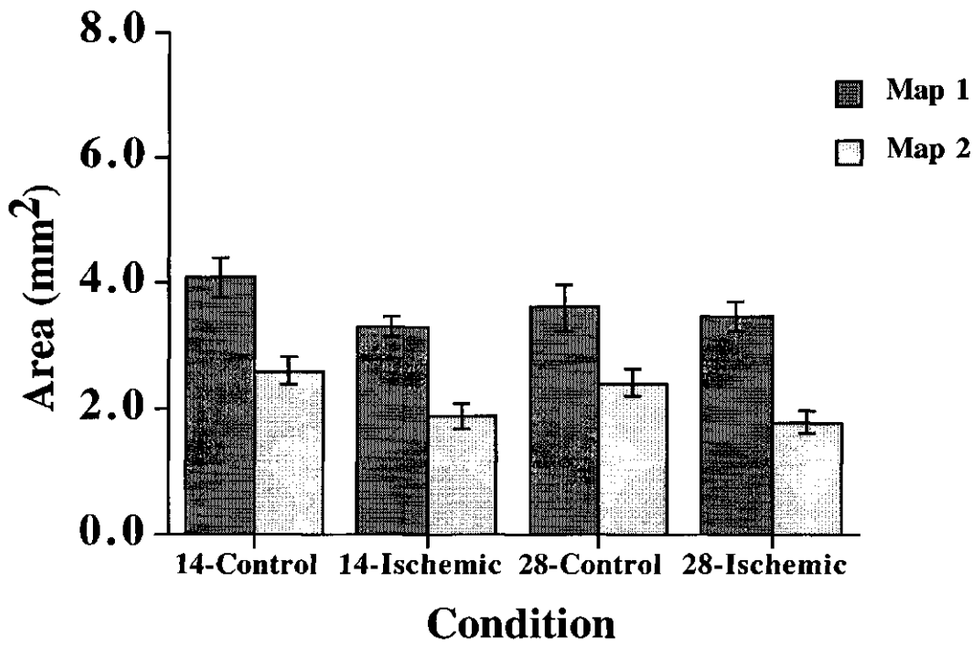

Figure 3.6 - Mean area (mm^2) for comparison of Map 1 after an ischemic infarct and Map 2 for fourteen-day (14-RH) Control and Ischemic Conditions and twenty-eight-day (28-RH) Control and Ischemic Conditions. There were no significant differences between 14-RH and 28-RH Conditions in either Control or Ischemic Conditions ($p<0.05$).

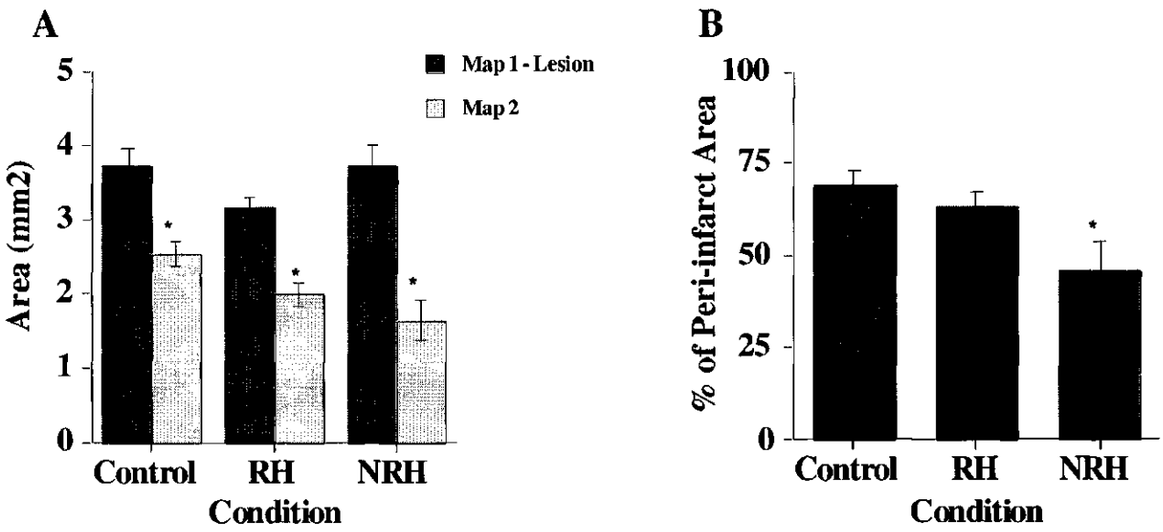

Figure 3.7 - Mean area (mm^2) for comparison of Map 1 -Lesion and Map 2 (A) and for the percent of peri-infarct representations in Map 2 (B). In all conditions the forelimb representations were significantly smaller (A, *$p<0.05$). The Non-rehabilitation (NRH) Condition exhibited significantly less peri-infarct representations than Control and Rehabilitation (RH) animals (B, *$p<0.05$).

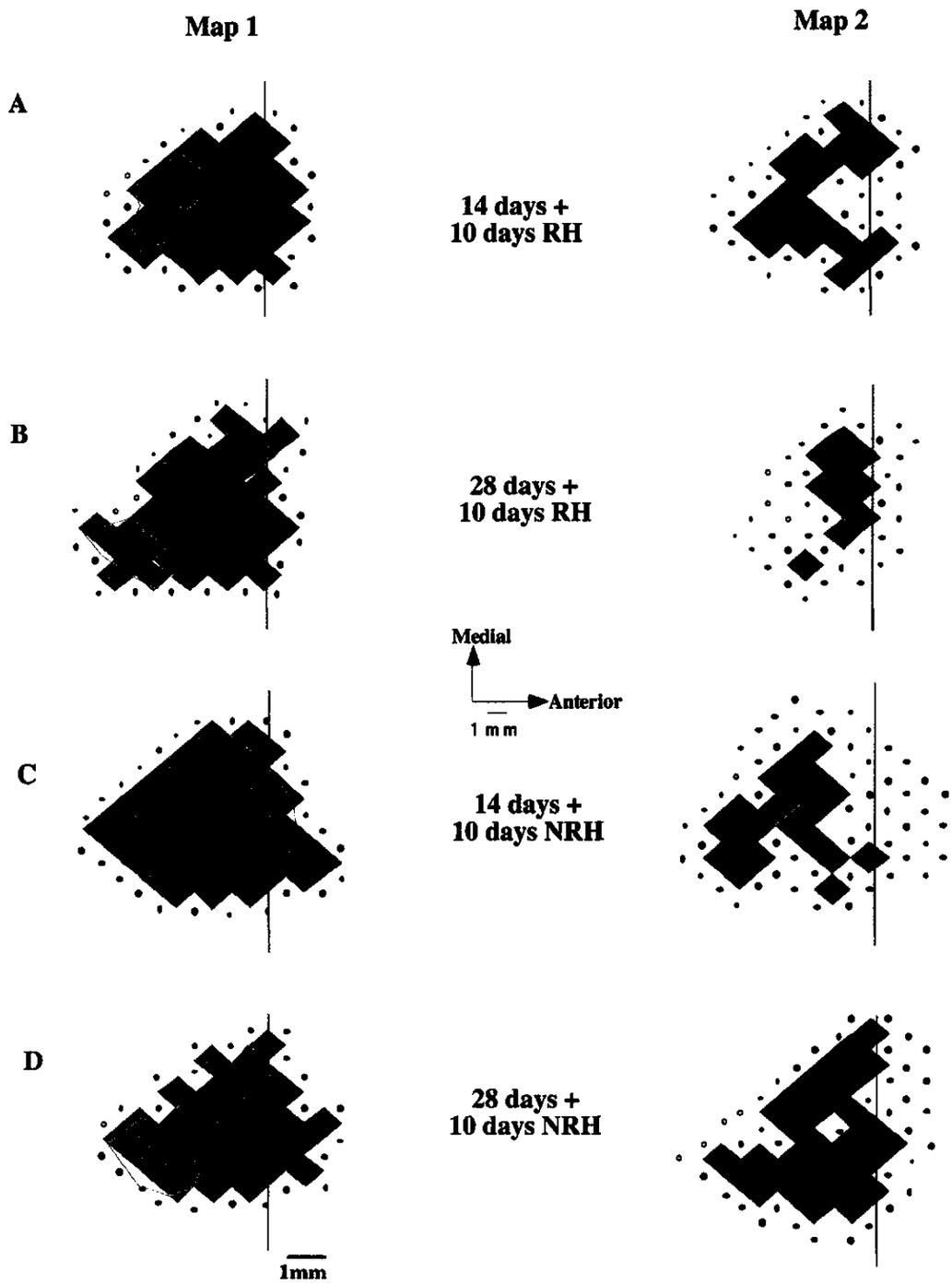

Figure 3.8 - Representative motor maps from 14-RH (A), 28-RH (B), 14-NRH (C), and 28-NRH (D) Conditions derived using ICMS. All four conditions exhibited a significant loss in forelimb movement representations in Map 2. Distal (digit/wrist) forelimb representations are depicted in green and proximal (elbow/shoulder) in blue. The ischemic area is denoted with a red circle and Bregma shown as a red line.

DISCUSSION

The present results extend previous work showing the alleviation of diaschisis with the administration of rehabilitation. More importantly, the results indicate that the effect of rehabilitation following stroke is beneficial independent of the duration of the delay following ischemia. Following the first mapping session, all ischemic animals exhibited a significant decrease in reaching accuracy. By the tenth day of rehabilitation, all but the Non-Rehabilitation (NRH) Conditions returned to pre-lesion accuracy levels. Animals that did not receive rehabilitation did significantly worse on the single pellet reaching task following the delay post-stroke. Surprisingly, all animals exhibited significantly smaller forelimb maps following rehabilitation. However, the Non-rehabilitation (NRH) had a significantly greater loss than the Control and Rehabilitation (RH) animals.

The significant decrease in map size in control animals was unexpected. Intracortical microstimulation is an invasive technique which requires exposing the cortex and repeatedly penetrating it with a microelectrode (Cheney, 1996). In previous studies we have shown decreases in the size of control representations following a delay after mapping (Goerzten et al., 2001; Kleim et al., 2002), however the difference has never been significant. The many penetrations may cause small amounts of damage and motor impairments. Damage causes lesion-induced plasticity such as hyperexcitability or decreased GABAergic inhibition (Witte, 1998; Mittmann and Eysel, 2001) not seen in the control animals. It has been hypothesized that brain damage may increase sensitivity to use-dependent compensatory neural growth (Schallert et al., 1997). In addition the amount of damage or size of the lesion may allow for different levels of lesion-dependent

plasticity and cortical reorganization (Nudo et al., 2000a). Therefore, the map size decrease in controls may occur because the amount of damage caused by the administration of ICMS may not be great enough to induce lesion-dependent plasticity as in ischemic animals.

Despite the decrease in map area in control animals, rehabilitation independent of delay of onset of administration reduced the magnitude of the loss of movement representations. Previous studies have shown that the most dramatic recovery occurs within the first thirty days following damage (Wade et al., 1985; Duncan et al., 1997). In addition, the most prominent physiological changes, such as hyperexcitability, occur approximately ten days following ischemia (Hagemann et al., 1998). This hyperexcitable property of the cortex has been proposed as the substrate for behavioral recovery and compensation (Witte et al., 1999). The increase in excitability may be due to a down regulation of $GABA_A$ receptors resulting in decreased GABAergic inhibition. When Layer IV was stimulated, GABA-mediated synaptic inhibition was weaker in the vicinity of the lesion suggesting the hyperexcitability of single neurons (Neumann-Haefelin et al., 1995). Excitability changes may also be due to NMDA receptor-dependent excitability increases surrounding the lesion (Buchkremer-Ratzmann et al., 1997). Changes in GABA and NMDA systems may facilitate behavioral recovery in response to rehabilitation following stroke due to the hyperexcitability of neurons and resulting lesion-induced plasticity.

Administration of rehabilitation two and four weeks following ischemia reduced diaschisis within peri-infarct cortex. Both groups that received delayed rehabilitation exhibited the same degree of loss of representations as control animals. We have

previously shown that peri-lesional disappearance of cortical representations was present twenty-four hours after injury (VandenBerg et al., 2001) and diaschisis was alleviated if rehabilitation was administered immediately following damage (Goertzen et al., 2001). Furthermore, in non-rehabilitation conditions there was a dramatic decrease in non-ischemic representations and associated motor decrements in reaching performance (Goertzen et al, 2001) consistent with this study. If the phenomenon of diaschisis was responsible for the depression of function within peri-infarct cortex, then rehabilitation would have served to retain peri-lesional representations as in the rehabilitation conditions.

The present results demonstrate that even delayed rehabilitation remains beneficial. In the absence of rehabilitation animals exhibited a greater decrease in performance that was associated with a greater loss of peri-lesional movement representations. Both NRH Conditions experienced a dramatic decrease in forelimb representation consistent with other studies (Nudo et al., 1996b; Goertzen et al., 2001). Although ischemia is associated with electrical changes in peri-infarct tissue, it may be necessary to perform rehabilitation at any time in order to make use of lesion-induced plasticity. Rehabilitation in the form of motor activity has been hypothesized as the means by which recovery of function is associated with a use-dependent growth and increase of spine density of pyramidal neurons (Jones and Schallert, 1994; Kolb et al., 1997). If neurons are hyperexcitable, it may lead to increased functional plasticity but rehabilitation is required to organize connections between neurons to form topographical representations within the motor cortex. It is important to note that even a delay in the

administration of rehabilitation utilized the increase in functional plasticity following ischemia.

The present experiment evaluated the effects of delayed rehabilitation of behavioral recovery and the organization of forelimb representations in rat motor cortex following a focal ischemic infarct. The results indicated that rehabilitation aids in behavioral recovery and alleviates peri-lesional dysfunction when delayed for two weeks or more. The results of this study provide further evidence that rehabilitation following stroke enhances functional and behavioral recovery and emphasizes the need for rehabilitative interventions even in the chronic phase of ischemic injury. This study provides evidence to support the idea that rehabilitation at any time is better than the absence of administration.

CHAPTER 4:

GENERAL DISCUSSION

This thesis addressed the etiology of peri-infarct diaschisis within the motor cortex and how delayed rehabilitation influences motor recovery and peri-infarct diaschisis. Specifically, it investigated changes in movement representations present in peri-infarct tissue in the acute and chronic phases of stroke. The loss of peri-infarct movement representations was linked to the occurrence of behavioral impairments. Two specific questions were addressed. First, how soon after ischemic insult does associative diaschisis appear? Second, do the benefits of rehabilitation have a critical window in order to get recovery and overcome diaschisis?

To address these issues, two experiments were performed. Experiment one addressed the question of how quickly the loss of movement representations occurred within peri-infarct cortex following stroke. Experiment two examined whether delayed rehabilitation facilitates behavioral and functional recovery following stroke.

MECHANISMS OF ASSOCIATIVE DIASCHISIS

This thesis demonstrated the presence of associative diaschisis within peri-infarct tissue twenty-four hours following stroke within the motor cortex. Diaschisis was proposed in the early 1900s as an explanation of exaggerated motor deficits caused by passive inhibition of intact areas of the brain in response to an injury (von Monakow, 1914). In recent studies, measures of diaschisis have been developed such as changes in cerebral blood flow and electrical properties of neurons. In this thesis, associative diaschisis is defined as the absence of forelimb movement representations within structurally intact areas of motor cortex following stroke. ICMS provides a direct

measure of the depression of function in intracortical connections. An explanation of how this form of diaschisis affects motor output can be derived from the method used to delineate representations within the motor cortex.

How Does ICMS Work?

Intracortical microstimulation (ICMS) was devised as a technique to uncover the functional organization of the motor cortex. The method of ICMS utilizes the intrinsic organization of the motor cortex. Microelectrodes are lowered approximately 1550 μm corresponding to layer V, the corticospinal cell layer. Pyramidal cells have an apical dendrite which extends toward the pial surface and numerous basal dendrites which project horizontally from the cell body. The axons of some layer V pyramidal cells form the corticospinal tract that passes directly from the cortex to the spinal cord (Porter et al., 1995). The application of a small amount of current through a microelectrode activates small clusters of pyramidal cells in the vicinity of the electrode (Cheney, 1996), which produces the overt movement response in the corresponding contralateral muscle. Current from the microelectrode activates horizontal afferents (Jankowska et al.,1975; Huntley et al., 1991). ICMS derived motor maps represent the most dominant output from a given locus, possibly reflecting the greatest density of corticospinal neurons that project to a majority of motor neurons of a given motoneuronal pool (Huntley et al, 1991). In most cases following intracortical microstimulation, latencies of descending volleys indicate that stimulation results in monosynaptic or polysynaptic activation of pyramidal tract cells (Jankowska et al., 1975).

Long horizontal collaterals of pyramidal cells are the mechanism by which representation zones may interact with each other to execute movement patterns (Keller,

1993). Clusters of pyramidal cells synapse with other pyramidal cells within the same representation zone (Keller and Asanuma, 1993). Groups of muscles are activated by intermixed columns of representational zones related to different muscles (Asanuma et al., 1967). Specific representations of muscles and evoked movements differ between animals and identical movements can be evoked from multiple, noncontiguous sites (Huntley et al., 1991). There are intrinsic bidirectional connections that connect one type of movement representation and representations of closely associated locations of the body (Huntley et al., 1991). The connectivity of axon collaterals provides inputs to many different movement representations and may be recruited during complex movements to coordinate the activity of motor cortical zones activating muscle groups synchronously (Huntley et al, 1991). There is an overlap of representations resulting from a mixing of cells with different target muscles in the same cortical territory as well as the fact that single cortical cells have multiple target muscles (Cheney, 1996). The spatiotemporal coordination of activity within individual representation zones may be mediated by intrinsic or intracortical pathways (Huntley et al, 1991; Aroniadou et al, 1993). Horizontal connections, which provide a substrate for intra-areal communication for muscular coordination, may mediate plasticity or dynamic adaptations (Sanes et al., 1997).

How are ICMS and Diaschisis related?

i. Energy Depletion

ICMS relies on the inherent structure of the cortex to produce reliable movement responses. If diaschisis is present following ischemia, functional representations may be altered. Because associative diaschisis is not present in peri-ischemic cortex one hour

post-ischemia, there may be a delay in the effects of the damaging processes present in the acute and subacute phases of stroke. For instance, the immediate cessation of blood flow to tissue during an ischemic insult causes an energy crisis which then drives secondary processes such excitotoxicity and peri-infarct depolarizations (Sapolsky, 1992b). Decreases in cerebral blood flow to levels lower than that required to sustain normal neuronal activity (Witte et al., 1997; Dirnagl et al, 1999) may take more than one hour to affect the organization of movement representations. Within peri-infarct tissue there is an increase in oxygen extraction, acidosis, high glucose utilization, and only residual levels of ATP consistent with energy depletion (Obrenovich, 1995). Protein synthesis is also inhibited (Kohno et al., 1994). Decreased protein synthesis has been linked to the immediate loss of movement representations in rat motor cortex (Kleim et al., 2002). Changes in neuronal activity may be the result of dysfunction of intracortical synapses within the peri-infarct area (Stroemer et al., 1995). Processes such as these result in delayed apoptotic cell death in peri-ischemic tissue (Dirnagl et al., 1999) in the absence of reperfusion or neuroprotective intervention. If brain tissue suffers an energy crisis then it is possible that it cannot support neural activity needed to produce overt movements following intracortical stimulation of layer V.

ii. Increased Excitability

Ischemia also induces changes in electrical activity of the motor cortex. It produces an excessive release of the excitatory neurotransmitter glutamate, which in turn overstimulates glutamatergic receptors, especially NMDA receptors (Zipfel et al., 2000), making tissue hyperexcitable. In addition, glutamate reuptake is impaired, so there is a prolonged activation of glutamate receptors (Dirnagl et al., 1999; Small et al., 1999).

Hyperexcitability in peri-infarct tissue leads to peri-infarct or spreading depressions (SDs). These transient waves of depolarizations suppress electrical activity with membrane depolarizations propagating in nonischemic regions (Lauritzen, 1994). They result from a loss of ionic gradients in which potassium and glutamate are expelled from neurons. In peri-ischemic tissue cells are healthy enough to repolarize but because of a massive amount of extracellular potassium and glutamate, the cell experiences continuous depolarizations for up to six to eight hours post-ischemia (Dirnagl et al., 1999). Stimulation to the motor cortex may be ineffective because hyperexcitability-related spreading depolarizations and ionic imbalances may prevent the propagation of action potentials to reach muscles in the contralateral body. However, because SDs are a transient phenomena, they do not account for the presence of diaschisis within the chronic phase.

iii. Increased Inhibition

Study of the vulnerability of pyramidal cells and interneurons in the hippocampus have shown that interneurons are less vulnerable to an ischemic event (Larsson et al., 2001). It is possible that when injured pyramidal neurons within peri-infarct tissue release excessive amounts of glutamate, they are overstimulating the more viable inhibitory GABAergic interneurons. Thus, there may be an increase in inhibition within the cortex resulting from an imbalance of GABA and glutamate. Other evidence supports the proposal that an imbalance in excitatory and inhibitory processes may result in organizational changes in the brain. There is evidence that LTP in the adult cortex can only be induced in the presence of GABA antagonists (Hess and Donoghue, 1994). Synaptic potentiation in the motor cortex has also been associated with an expansion of

movement representations (Teskey et al., 2001). So, increased GABA resulting from an initial dysfunction of pyramidal cells in the form of glutamatergic synapses, might first impair LTP-like changes in the cortex and prevent stimulation induced expansion of movement representations. This supports the occurrence of the failure of map size to increase in peri-ischemic tissue after one hour. Over time the loss of glutamatergic synapses may lead to an eventual decrease in movement representations (Goertzen et al., 2001) associated with an ischemic event.

ICMS and diaschisis exert influences on the same structural characteristics of the motor cortex. Shortly after a lack of blood flow, energy is depleted and therefore neurons are unable to perform basic cellular functions. Also, the balance of excitatory and inhibitory actions of single neurons and groups of neurons is disrupted preventing the proper response following microstimulation. Finally, differential vulnerability of interneurons may support a shift from primarily excitatory synapses to a more dominant inhibitory control over cortical organization. This evidence supports the finding of this thesis that there are diaschitic decreases in map size twenty-four hours following an ischemic event.

How does diaschisis contribute to motor impairments?

It has been shown that diaschisis-induced dysfunction of intracortical connections within the motor cortex can cause abnormal electrical activity within the cortex. How does this specifically relate to behavioral abnormalities exhibited following an ischemic event? Two investigations directly examined the mechanisms by which diaschisis may control behavior so as to cause motor impairments. These investigations have shown that

either exaggerated cortical inhibition of pyramidal tract neurons or disturbed synaptic connections may be responsible for motor impairments.

One study (Classen et al., 1997) used transcranial magnetic stimulation (TMS) to investigate inhibitory axons within motor cortex proposing that motor deficits were caused by excessive cortical inhibition of pyramidal tract neurons. Abnormally long silent periods (SPs) following stimulation or increased cortical inhibition was associated with a clinical syndrome in patients which included symptoms of muscular weakness, disturbed voluntary and spontaneous movements, and decreased ability to maintain a constant force. The authors propose that extremely long SPs could result from sustained firing of inhibitory interneurons. Specifically, SPs may be caused by a defect in mutual inhibition between GABAergic interneurons, by pathologically enhanced excitatory input to interneurons, or decreased inhibitory control of them (Classen et al., 1997). The results from this experiment suggest that motor deficits are due to the dysfunction of intracortical afferents. If hyperexcitability within peri-infarct tissue results in the overstimulation of inhibitory interneurons, then it is plausible to suggest that motor deficits associated with diaschisis are the result of intracortical connection properties. Finally, there was a co-occurrence of exaggerated SP duration and motor neglect and SP normalization was associated with clinical improvement (Classen et al., 1997). Therefore, diaschisis related increases in inhibition are related to a documented clinical syndrome.

The second experiment studied the effect of ischemia on synaptic efficacy and axonal conductance. The authors hypothesized that the failure to recover from motor impairments may be due to unsatisfactory restoration of synaptic activation within the

cortex and/or the blockade of electrical impulses in subcortical areas (Bolay and Dalkara, 1998). In a transient middle cerebral artery occlusion (MCAO) model, motor dysfunction seemed to be caused by transmission failure despite recovered axonal conduction (Bolay et al., 1998). This may account for prompt motor recovery following ischemia. When recording SEPs in the forelimb area of sensorimotor cortex following reperfusion, recovery was incomplete, possibly due to persistent damage to synapses (Bolay et al., 1998). The authors concluded that pyramidal motor function was rapidly lost following ischemia due to a loss of excitation in the cortex and blockage of axonal conduction in subcortical regions. They also concluded that axonal conduction recovers shortly after reperfusion, but motor dysfunction persists along with synaptic transmission defects within the motor cortex. Therefore, diaschisis related motor impairments utilize the laminal organization of the motor cortex and the inherent electrical and synaptic connections within intact cortex to produce motor syndromes not explained by the direct damage itself. These findings are consistent with von Monakow's proposal in the early 1900s.

Can Diaschisis be Overcome with Rehabilitation?

In the previous section, energy failure, increased excitability, and increased inhibition were highlighted as major determinants of diaschisis-related motor deficits following stroke. To overcome diaschisis-related motor impairments many rehabilitative strategies have been developed, however, little is known about the mechanisms that facilitate the resolution of diaschisis and subsequent behavioral recovery.

Many studies have shown that following stroke if rehabilitation strategies are administered there is a massive increase in activation of the brain (Chollet et al., 1991;

Weiller et al., 1993; Cramer et al., 1997; Liepert et al., 2000) associated with improved motor function. Specifically, activation is unbalanced between the affected and unaffected hemispheres (Traversa et al., 1997). The cellular mechanisms underlying the extension of motor areas in rapid occurrence suggest that there is a reinforcement of existing circuits with the administration of rehabilitation and that GABA-mediated inhibition induced by ischemia could facilitate cortical spread of activation (Chollet and Weiller, 1994). Also present is an increase in regional cerebral blood flow (CBF) (Chollet et al., 1991) which is thought to reflect the discharge rate at the presynaptic terminal underlying skill acquisition (Grafton et al., 1992). Enhanced metabolic interactions in motor circuitry were found to be predictive of motor recovery early after stroke (Cramer et al., 2000a). In addition, motor evoked potentials improve reflecting better synchronization of cortical motor neurons and related corticospinal output (Traversa et al., 2000). These results suggest that there is an increase in the excitability of neurons innervating or an increase in the excitability of neuronal tissue in the infarcted hemisphere or both (Liepert et al., 2000). It is likely that this mechanism involves a decrease in inhibitory activity of local interneurons therefore unmasking pre-existing excitatory connections (Jacobs et al., 1991).

Rehabilitation leads to the recruitment of large numbers of neurons for the innervation of movements of the stroke-affected extremity adjacent to those involved before therapy (Liepert et al., 2000). Normalization of activation and CBF reflects an increase in synaptic efficacy in which there is a decrease in excitation of neuronal connections without a deterioration of function (Liepert et al., 2000). Learning and practicing of a motor skill is accompanied by an increase in the efficacy of horizontal

connections in the motor cortex and the plasticity of these connections could contribute to reorganization of motor cortex representations that accompanies motor skill learning (Rioult-Pedotti et al., 1998). Cortical areas in the damaged hemisphere may contain representations of movement and these may access the spinal cord via alternated pathways that bypass the lesioned pyramidal tract (Chollet et al., 1994). Functional reorganization occurs in the damaged hemisphere, supporting the idea that cortical areas ipsilateral to damage participate in the recovery process (Weiller et al., 1993). Synaptic plasticity of cortical horizontal connections underlies cortical map reorganization (Hess et al., 1994; Nudo, 1999). Horizontal collaterals terminate on nearby local circuit neurons forming inhibitory synapses onto pyramidal neurons and these contacts provide local feed-forward inhibition (Afifi and Bergman, 1998). Organization of connections within the motor cortex predict that a decrease in inhibition would tend to strengthen coupling between reciprocally connected pyramidal cells and may underlie modifications in motor cortex output that could produce new motor output architectures (Sanes et al., 1997). It appears that the connectional substrate for reorganization is already present within primary motor cortex and that new maps may emerge when the balance of excitatory and inhibitory connections are changed (Sanes et al., 1997). The motor map reorganizes by changing the effectiveness of outputs to remaining effectors when access to preferred effectors is blocked by removal or lesions (Kaas, 1991).

LIMITATIONS

Any study using an experimental model to study natural phenomenon will have some limitations or confounding variables. In this thesis, the first limitation is the changes in representation size altered by intracortical microstimulation. Second, the age

of the animals used may be considered a confounding variable. Finally, the model of ischemic insult itself may present some limitations in studying stroke.

In experiments one and two of this thesis, intracortical microstimulation (ICMS) was shown to change the properties of the motor cortex. In experiment one, ICMS caused an expansion of forelimb representations one-hour following stimulation. In experiment two, control animals exhibited a significant decrease in caudal forelimb area representations. These results are consistent with other studies showing facilitation of movement representations expand the borders of these representations (Brown et al., 1912; Nudo et al., 1990). Shrinkage of cortical representations following ICMS alone has been reported, although decreases in representation area have not previously been significant (Goertzen et al., 2001; Kleim et al., 2002). On the other hand, ICMS allows the direct measurement of specific functions of the brain following environmental manipulations. Few measurements allow this type of direct observation of changes in the brain. For this reason, the method of intracortical microstimulation remains an effective means of evaluating the functional state of the motor cortex following ischemia.

Second, the age of the animals used in this study is not directly translatable to the human problem of stroke-induced impairments and recovery. In experiments one and two, the rats used were approximately ninety days at the onset of experimentation. The rats at this time are considered young adults, however, many people experience strokes in the later stages of life (Wade et al., 1985; Classen et al., 1997). Animals of this age are used because we believe that the mechanisms following ischemia in young and old rats are the same. This model allows us to see ischemia-induced mechanisms at a magnified level because of the level of functioning in young adults. Because the mechanisms

following stroke are similar at any age, the results of experiments one and two are translatable to older animals and to the human population.

Finally, the stroke model chosen may present some limitations. The model used in this thesis mimics the effects of a stroke via coagulation of surface vasculature within a focal, physiologically defined area of the rodent motor cortex. This method of ischemia does not reflect the extent of damage that most patients experience following a stroke in which a larger area of the brain experiences a loss of blood flow. The purpose of this thesis was to investigate the functional changes in tissue surrounding a permanent, focal ischemic infarct. This method of ischemia provides us with the technique to administer damage to a confined area of the cortex and measure the direct consequences in function in surrounding areas. In this manner ischemia via coagulation of surface vasculature allows us to answer the questions put forth in the two experiments.

Although the method of investigation used in this thesis may present some confounding variables, the benefits of their use outweigh the disadvantages. Intracortical microstimulation provides the manner in which the function of specific brain areas can be studied. Younger animals also provide a useful technique. Finally, the stroke model used also us to cause damage within a specific area and thus cause specific impairments. All three techniques allow us to translate the findings of this thesis to the human population and allow us to build on evidence in stroke research and behavioral recovery.

IMPLICATIONS

The findings from this thesis indicate that ischemia results in the loss of movement representations within one day and delayed rehabilitation is effective in overcoming diaschisis and improving function. The goal of experiments such as these is to characterize the physiological processes occurring in ischemic tissue both immediately after insult and during rehabilitation. It also reinforces the idea that rehabilitation mediates both functional and behavioral recovery.

The results from Experiment 1 show there are changes that occur within the cortex within twenty-four hours. The second experiment showed that delayed rehabilitation is beneficial even up to one month. Furthermore, rehabilitation dependent improvements in motor ability were accompanied by an increase in peri-infarct movement representations. It is hypothesized that changes within intracortical connections of the motor cortex have been provided as a possible mechanism by which intact areas of the motor cortex compensate for the loss of function within neighboring areas.

Most studies report that rehabilitative interventions are administered following initial evaluation (Cote et al., 1986; Cramer et al., 1997; Jorgensen et al., 1999) or on arrival to the stroke unit (Jorgensen et al., 2000). This thesis shows that delayed rehabilitation is still effective at one month. Implications of the timing of rehabilitation may be applied to the clinical setting.

It would be useful for future studies to determine what processes are present immediately after stroke so that pharmacological interventions in use today may be made more effective. It would also be useful to examine what therapeutic strategies are most

beneficial and at what time point they should be administered. The results in this thesis serve to expand diaschisis research by demonstrating how quickly diaschisis in peri-infarct cortex occurs and the benefits of delayed rehabilitation for recovery following stroke.

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