UNIVERSITY OF CALGARY

Experimental Infarct Mitigation with Hyperoxia at Normobaric Pressure

by

Erin Patricia Flynn

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE

DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF NEUROSCIENCE

CALGARY, ALBERTA

MAY, 2000

Abstract

The focus of this thesis is the effects of high blood oxygen levels during and after ischemic stroke, in view of popular theories of damage in the reperfusion period due to an increased production of oxygen-derived free radicals.

Five groups were studied: (1) intra-ischemic normoxemia, (2) low intra-ischemic hyperoxemia, (3) high intra-ischemic hyperoxemia, (4) reperfusion hyperoxemia, and (5) intra-ischemic plus reperfusion hyperoxemia. Male Wistar rats were subjected to a 60 stroke induced by an intraluminal suture.

All hyperoxemia treatment groups fared better, both behaviorally and neuropathologically, than the normoxemic control group. Both levels of intra-ischemic hyperoxemia, and reperfusion hyperoxemia, resulted in improved Bederson neurologic scores, reduced cortical infarction and less total damage. The intra-ischemic plus reperfusion hyperoxemia treatment produced the most favorable results, with drastic improvements in the measured variables.

It is concluded that increased blood oxygen levels during and after stroke are not damaging, but rather advantageous.

Acknowledgements

The following thesis would not have been possible without the scientific and intellectual guidance of Dr. Roland N Auer. While I may have done all of the grunt work, Dr. Auer came up with the most important part of this thesis – the idea that elevated blood oxygen levels may be an effective therapy for ischemic stroke.

The work in this thesis also would not have been possible without the help of Dr. Dubravka Rakič. Although she has a way of making everything look deceptively easy, Dubravka taught me how to do every surgical procedure needed for this thesis. She also provided valuable computer information, and, every once in a while, a shoulder to cry on.

I would also like to thank my committee members, Dr. Quentin Pittman and Dr. Keith Sharkey, who provided information and ideas on things I otherwise may not have considered. Their input was extremely valuable.

The graduate coordinator, Dr. Ray Turner, was also very important to the completion of this thesis. In addition to guiding me through the administrative aspects of being a graduate student, he also helped provide funding which allowed me to continue to eat.

Dedication

This thesis is dedicated to my parents, Rand and Linda Flynn, who taught me from a very young age how important education is. Without their constant support, both emotionally and financially, I would never have made it this far, and if it weren't for their consistent encouragement, I would still be slinging drinks in a bar.

TABLE OF CONTENTS

List of Tables

List of Figures

CHAPTER ONE: INTRODUCTION

Stroke is a brain injury that affects many individuals. In Canada, 50,000 people a year have a stroke, one third of which are fatal. Successful clinical treatments for stroke are limited to only one measure at present, thrombolysis, despite the fact that stroke research on animals has revealed several determinants of stroke size. Factors such as neurotransmitters, temperature, glucose, and blood pressure have all been shown to affect the size of brain damage in rodents after stroke. The research that will be presented in this thesis represents a novel potential treatment for stroke – namely the elevation of blood oxygen levels. I will begin by reviewing the factors that have been shown to affect brain damage after stroke. Then, experiments that have already varied oxygen during and after stroke will be reviewed. Because high oxygen has been proposed and proven to have pathological effects, the concepts of reperfusion injury and oxygen toxicity will be examined. The characteristics of oxygen in the atmosphere and the blood will then be given to help comprehend the beneficial effects of elevated blood oxygen during a stroke. I will then describe in detail the experiments completed for this thesis. The results of the experiments, some of which are somewhat surprising, will then be presented, followed by the conclusions which can be drawn based on the results. Possible shortcomings of this research, together with future experiments this research leads to, will be presented at the end of this thesis.

The first concept which needs to be addressed is the definition of stroke. What happens inside the brain when a person suffers a stroke?

What is Cerebral Ischemia?

Blood supply to the brain is principally derived from two pairs of arteries. The

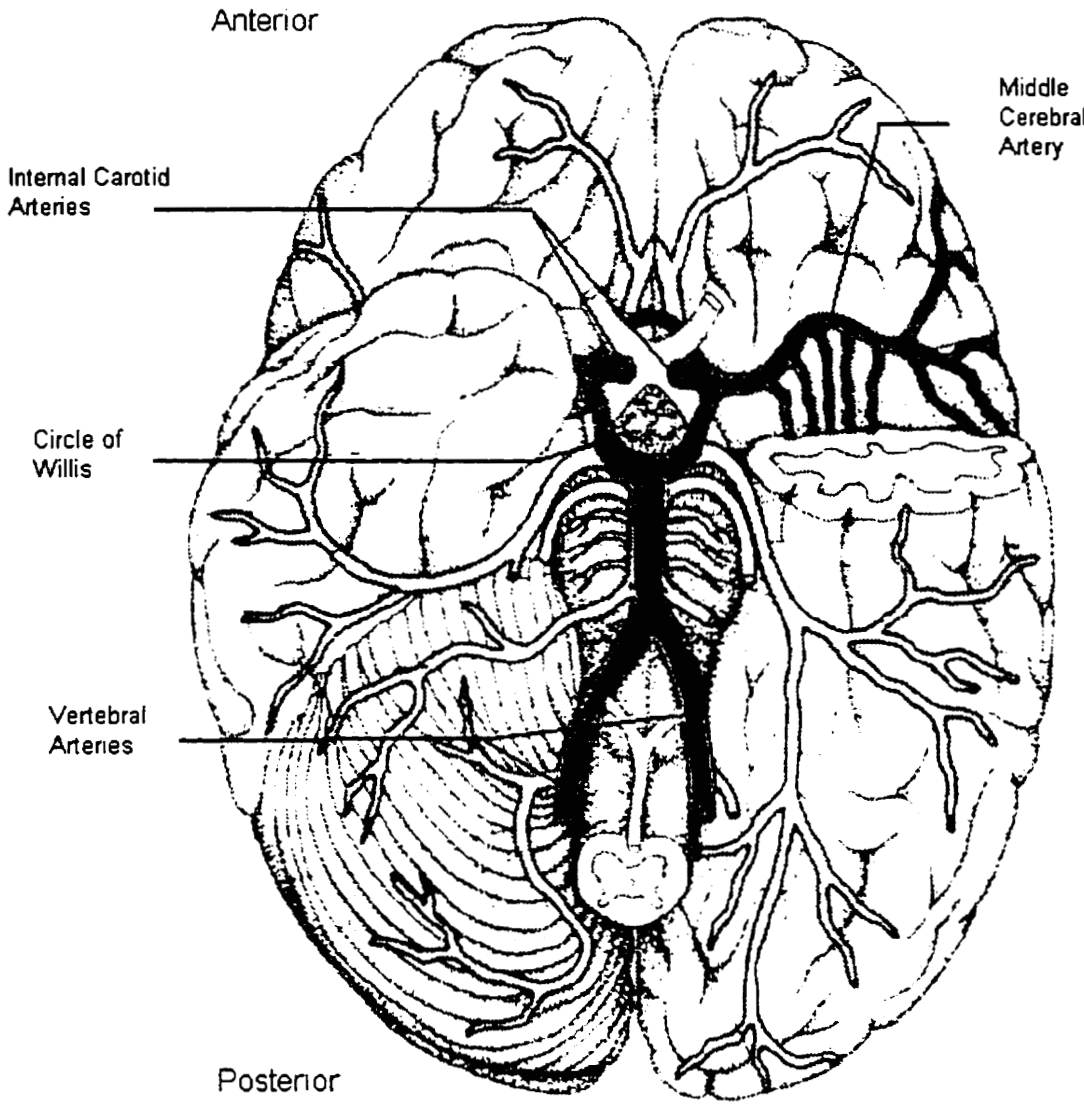

Figure 1 - Principle blood supply to the brain. Inferior view of a human brain. The arteries composing the Circle of Willis are colored in pink. Adapted from Diamond et al, 1985.

anterior circulation is fed by the left and right **internal carotid arteries,** and the posterior circulation is fed by the left and right **vertebral arteries**[1]. The anterior and posterior circulations do flow together, being linked at the base of the brain by a network of interconnected arteries, or an **anastomosis**, called the **Circle of Willis**[2] (figure 1).

When a person has a stroke, adequate blood supply to the brain is disrupted due to a blockage within a blood vessel. This reduction in blood supply is known as **ischemia**

(from Greek: *ischo*, to hold back + *haima*, blood)[3]. The reduction of blood can be the result of a thrombosis (a stationary clot), an embolism (a floating clot that has become lodged in a smaller vessel), or hemorrhage (rupture or leaking of blood from vessels)[4]. If a blockage occurs within or near the Circle of Willis, the connection of the carotid and vertebral arterial systems may provide adequate compensatory blood flow to the cerebral hemispheres. In the event of inadequate blood supply, the tissue normally supplied with blood becomes **necrotic**, or dead. The affected area is known as an **infarct**.

Global cerebral ischemia is a term used to describe what happens when the entire brain is ischemic. This occurs in humans when the heart stops beating and blood flow of the entire body ceases. **Focal** cerebral ischemia is a term used when only a discrete area of the brain is deprived of blood, as occurs when one has a stroke. These two brain insults are pathogenetically different, and should be researched and treated as two independent forms of brain damage.

Cell Death in Ischemia

Selective Neuronal Necrosis vs. Pan-Necrosis

Damage to the brain occurs in one of two forms, depending on whether the damage affects neurons only, or whether other elements of neural tissue are also damaged. **Selective neuronal necrosis**, or incomplete necrosis, is the term used when only neurons are damaged. If all elements of the affected tissue are damaged, including neurons, glia, and blood vessels, the lesion is called **pan-necrosis**, or complete necrosis[3]. The potential for recovery is far greater for selective necrosis than for pan-necrosis because some neural elements remain undamaged in the former. Pan-necrosis results in fluid filled cysts where neural tissue once existed[3].

The term **penumbra** is one borrowed from astrology, where it refers to the vaguely shaded annulus that encompasses the moon during a solar eclipse[5]. In neuropathology, the term penumbra refers to a very thin band of selective neuronal necrosis which surrounds the dense area of pan-necrosis after an ischemic insult[5]. The commonest explanation for the penumbra is that it is a brain region that has experienced a reduction in blood flow (hypoperfusion), but has still experienced enough vascularization to preserve some of the cells. The severity of ischemia diminishes with distance from the infarct core, and the penumbra represents an area of "bare survival" of glia and blood vessels[5].

Glutamate Excitotoxicity

Theories of why neurons die after a period of ischemia involve neurochemicals. **Glutamate** and aspartate are the major excitatory amino acid neurotransmitters of the Central Nervous System (CNS). They account for most of the fast synaptic transmission that occurs between neurons[6]. The fundamental idea that neurons can be damaged by overstimulation, or **excitotoxicity**, was originated by Olney with his observations on glutamate toxicity[7-9].

The association between glutamate excitotoxicity and ischemic necrosis arose out of the observations that both phenomena produce necrosis of only certain neurons in distinct brain regions[10]. This concept is known as **selective vulnerability**[3]. The similarity of selective vulnerability in both excitotoxic necrosis and ischemic necrosis gave rise to the formation of the excitotoxin hypothesis for ischemic injury. This hypothesis states that the selective vulnerability seen in ischemia is due to excessive increases in the concentration of excitatory amino acids, and subsequent excitotoxicity[10].

Measurements of amino acid concentrations by intracerebral microdialysis during ischemia have shown that glutamate and aspartate concentrations do indeed increase during ischemia[11]. As a result of this increase in extracellular glutamate, receptor-coupled ion channels are opened and a large influx of **calcium** enters the cell. This calcium influx activates a host of catabolic enzymes that ultimately cause neuronal death[10]. In an effort to test this hypothesis, Benveniste measured extracellular glutamate concentrations during ischemia, evaluated the toxicity of any glutamate concentration increase, and related any glutamate increase to calcium homeostasis. It was found that toxic levels of glutamate were produced during ischemia, and that increases in extracellular glutamate correlated with decreases in extracellular calcium, thus supporting the glutamate excitoxic theory of ischemia[10].

Glutamate does not act on a single receptor, but rather at multiple receptors which are often co-localized on neurons[12]. Glutamate receptors are classified into three groups: 1) N-methyl-D-aspartate (NMDA), 2) (AMPA)-kainate, and 3) metabotropic[13]. Studies that have attempted to mitigate ischemic injury with glutamate antagonists have been successful in animal models. Simon et al[14] found that microinfusion of an NMDA antagonist into the hippocampus before ischemia protected against the development of ischemic damage. Buchan et al[15] found that intraperitoneal injections of an AMPA antagonist also reduced infarct size. Testing of a metabotropic glutamate receptor antagonist revealed that the inhibition of these receptors also resulted in neuroprotection from ischemia[16].

Clinical Drug Trials

Based on this preliminary success in animal models, clinical trials have tested whether drugs which affect neurotransmission can mitigate damage arising from cerebral ischemia in humans. Although the results are not published, drug trials have been uniformly unsuccessful at treating stroke. Thus far, over a dozen clinical drug trials have been completed, most agents aiming to protect the brain parenchyma, or neural tissue.

The inconsistent results between animal and human trials can be at least partially understood in light of a study done by Nurse and Corbett[17]. They found that the glutamate antagonist NBQX, which was shown to protect hippocampal CA1 neurons against global ischemia[18], also produced a period of subnormal brain temperature. Hypothermia has been shown to provide neuroprotection after ischemia (see below). When the post-ischemic brain temperature of the NBQX gerbils was regulated to normothermia, no neural protection was found[17]. Based on these results, they concluded that an induced period of hypothermia brought on by a drug could obscure the interpretation of pre-clinical drug trials.

One chemical agent has had success in reducing stroke size at both the animal and human level. If the occlusion of an artery that causes ischemia is due to a blood clot, as opposed to a cholesterol or tissue clot, it is conceivable that a blood clot dissolving agent would lessen the degree of ischemia. The severity of ischemia is related to the duration of occlusion (see below). Zivin et al[19] found that intravenous administration of tissue plasminogen activator (t-PA), an agent that dissolves the fibrin network which makes up a blood clot[20], immediately after the injection of numerous small blood clots into the carotid circulation of rabbits caused a significant reduction in neurological damage.

Marler et al[21] found the same improved neurological outcome when t-PA administration was tested in a human clinical trial.

While excitotoxicity may be a mechanism of neuronal death after ischemia, brain chemical therapy should not be the only treatment considered for stroke. The optimal management of clinical ischemia may involve the manipulation of physiological parameters in addition to, or instead of, drug administration. Animal trials that have varied temperature, blood glucose level, blood pressure, ischemic duration, and blood oxygen levels during and after cerebral ischemia have shown that these parameters do in fact influence infarct size. A brief review of this literature will now be presented.

Temperature

Cooling of the brain during ischemia has been known for at least 50 years to provide neuroprotection[22-25]. Experiments done in the last 20 years provide overwhelming evidence of the validity of early experiments. While early experiments decreased the core body temperature, Busto et al[26] allowed brain temperature to vary independently of core body temperature during ischemia in rats. They found that the core body temperature unreliably reflects brain temperature during ischemia, and that small decrements of intra-ischemic brain temperature (2-5°C) markedly mitigated the histopathological changes following 3-day survival. These results of **hypothermia** during ischemia have been supported in both global stroke models, where the entire brain is ischemic[27-30], and focal ischemia models[31-34], where only a portion of the brain is ischemic.

Cooling of the brain temperature after ischemia has also been shown to have neuroprotective properties. While brief periods (2-3 hours) of postischemic hypothermia

have had mixed results, producing either reduced damage[35-37] or no change in damage[29,38,39], longer periods (12-24 hours) of postischemic hypothermia have consistently shown improved neurological outcome[17,40-42].

As a corollary to the beneficial outcome of hypothermia with ischemia, **hyperthermia**, or elevated temperatures, has been shown to produce deleterious effects with ischemia. Dietrich et al[43] compared intra-ischemic normothermia (37°C) to intra-ischemic hyperthermia (39°C). They found that hyperthermia not only increased mortality and injury, but also resulted in more frequent injury. Other studies have verified these findings in both global[44,45] and focal[46] stroke models. Hyperthermia induced after an ischemic period has also been shown to aggravate ischemic injury[47]. Interestingly, hyperthermia induced 24 hours before an ischemic period was found to make the damage less severe[48], probably by inducing protective, heat shock proteins.

Because of the powerful influence brain temperature has on ischemic injury, it is of utmost importance to consider and control for this variable during a stroke experiment to avoid a confounding variable.

Blood Glucose Level

Another physiological parameter which has been shown to influence infarct size after a stroke is blood glucose level. It has been shown that inducing **hyperglycemia**, or elevated blood glucose levels, above 10-13 mM after cerebral ischemia caused more seizures to develop and greater histological damage when compared to a normoglycemic control group (3-9 mM), and hyperglycemia above 16 mM caused seizures which were invariably fatal[49]. The exacerbating effects of hyperglycemia on ischemia have been found by other researchers as well[50-53].

The effects of **hypoglycemia**, or lower than normal blood glucose levels, have been shown to be beneficial after cerebral ischemia. Adult rats which were fasted for 48 hours prior to ischemia showed reduced cerebral necrosis when compared to a non-fasted group, suggesting than low blood sugar levels may mitigate ischemic necrosis[54]. New-born rat pups who were fasted showed this same reduction in ischemic necrosis[55].

A popular method of inducing hypoglycemia is to inject insulin, a protein hormone secreted from the pancreas that increases the uptake of glucose and amino acids by most tissues, thus lowering blood glucose level[56]. Initial studies which used insulin to reduce blood glucose levels after global ischemia indicated that insulin administration could reduce ischemic necrosis[57].

To determine whether the neuroprotective effects of insulin in global ischemia is due to a direct effect on the brain or an indirect effect of reducing blood glucose level, stroke experiments were undertaken in which insulin was administered alone or at the same time as glucose. A third control group experienced ischemia with saline injection. The results indicate that insulin has a direct effect in mitigating neuronal necrosis after global ischemia, whether or not hypoglycemia is allowed to occur[58]. Additional experiments were performed to further test whether insulin interacts directly with brain parenchyma by injecting insulin directly into cerebral ventricles. It was shown that insulin injection reduced ischemic necrosis, suggesting that insulin does indeed have a direct neuroprotective effect on CNS parenchyma[59].

Insulin administration has also been shown to have a mitigating effect on infarct size after focal ischemia[60]. Unlike global ischemia, glucose administration at the same time as insulin administration nullified most of the neuroprotective effects, suggesting

that the protection insulin provides with focal ischemia is due to a reduction in peripheral blood glucose levels and not due to a direct effect on CNS parenchyma[60]. The analysis of blood glucose levels demonstrated that blood glucose levels correlated with infarct size after focal ischemia, regardless of whether insulin was injected, supporting the notion of neuroprotection due to hypoglycemia in focal ischemia[60].

Since blood glucose levels are influential on ischemic injury, it is also important to control for and to consider this variable during stroke research if the results of experiments are to be fully understood.

Blood Pressure/Ischemic Intensity

Blood pressure during a stroke has also been shown to greatly influence infarct size[61]. Zhu and Auer found that subjecting male rats to graded **hypotension**, or decreased blood pressure, during a period of focal ischemia produced infarct sizes that got larger as blood pressure got lower. This can be understood by considering that lower levels of blood pressure could collapse microvasculature around the ischemic area, thereby decreasing collateral circulation and increasing the degree of ischemia. Analogous to Zhu and Auer's findings, Drummond et al[62] found that inducing **hypertension**, or elevated blood pressure, in rats during focal cerebral ischemia improved local blood flow and reduced ischemia. The beneficial effects of hypertension during ischemia has also been displayed in experimental primate focal ischemia[63]. This explains the adaptive value of Cushing's response, the spontaneous elevation of blood pressure in response to acute cerebral ischemia[64], or other brain injuries. It is important to note, however, that hypertension may result in increased edema[65] and increased hemorrhage[66], factors which may cause necrosis and mortality secondary to the ischemic

insult.

Because of the observed and conceived effects that variations in blood pressure may cause with ischemia, this is also a variable which must be monitored and considered when analyzing the results of experimental stroke.

Related to, but different from, blood pressure is the amount of blood reaching ischemic tissue. This ischemic density has been shown to influence infarct size. Steen et al[67] compared complete ischemia to incomplete ischemia, and concluded that some blood flow results in better neurological outcome than no blood flow. For this reason it is important to occlude the relevant artery to the same degree when conducting stroke research.

Ischemic Duration

The duration of ischemia has also been shown to influence infarct size. Zhu and Auer[61] found that at all three levels of blood pressure tested, infarct size increased with every additional 20 minutes of focal ischemia. It has also been shown that interrupting a period of focal ischemia with intermittent reperfusion, effectively breaking up the ischemic period into smaller periods, also has beneficial effect[68]. For these reasons it is important to keep the duration of ischemia constant in stroke research.

Ischemia and Oxygen

Hypoxia vs. Ischemia

Brain damage due to stroke is often incorrectly referred to as "hypoxic/ischemic brain damage" in the literature[69,70]. During ischemia, important substances in the blood, including oxygen and glucose, are not delivered to the tissue. As well, metabolic waste products arising from cellular respiration, such as lactate and H^+ ions, are not removed

from the tissue. Ischemia is therefore fundamentally different from **hypoxia**, which is a lower than normal level of dissolved oxygen in the blood, while blood flow is actually increased[71]. Because of these obvious differences, inadequate blood flow and low blood oxygen levels should be researched and treated as two fundamentally different phenomena.

Hypoxia and Hyperoxia during Ischemia

Miyamoto and Auer[70] set out to isolate the effects of hypoxia and ischemia by varying the arterial blood oxygen levels into the low, normal, and high range during focal ischemia in male Wistar rats. They found that a very low blood oxygen level (**hypoxemia**) (PaO_2 = 25 mm Hg), even at a low blood pressure (30 mm Hg), did not, by itself, produce necrotic neurons. The tissue is probably saved due to the compensatory mechanisms in the brain that increase blood flow. Behavioral evidence supports this finding – mountaineers climbing at high altitudes frequently display errors in judgement not seen at lower altitudes. However, detailed neuropsychological tests done on mountaineers 2-7 weeks following their return from base camp after a Mount Everest expedition reveal no permanent neuropsychological impairment[72], suggesting that even severe hypoxia is not accompanied by permanent brain damage.

Hypoxemia (PO_2 = 46.5 ± 1.4 mm Hg) induced at the same time as ischemia, however, was found to exacerbate the necrosis from 16.6 ± 7.0% to 24.3 ± 4.7% of the hemisphere, when compared to a normoxemic (PO_2 = 120.5 ± 4.1 mm Hg) control group. This finding suggests that arterial oxygen variations in the low range can influence the infarct size produced by a lack of blood flow. Miyamoto and Auer also varied arterial blood oxygen levels into the high range. They found that elevating blood

oxygen levels (**hyperoxemia**)(PO_2 = 213.9 ± 5.8 mm Hg) at the same time as inducing cerebral ischemia mitigated the infarct size to only 7.5 ± 1.9% of the hemisphere. This finding suggests that arterial oxygen variations in the high range can also influence the infarct size produced by a lack of blood flow.

These results of the general analysis of damage in the entire hemisphere were sharpened when the cerebral neocortex was examined. In the cerebral neocortex, hypoxemia, normoxemia and hyperoxemia during ischemia produced necrosis of 12.8 ± 3.1%, 8.0 ± 4.6%, and 0.3 ± 0.2% of the hemisphere, respectively ($p<0.01$). Cortical infarction was eliminated entirely with hyperoxemia during ischemia in 8 of 10 rats. Based on these provocative results, Miyamoto and Auer concluded that while intra-ischemic hypoxemia exacerbates ischemic necrosis, intra-ischemic hyperoxemia could potentially mitigate brain damage, especially in the cortex. Because no hyperbaric oxygenation was used, the results could have wide clinical applicability.

Because the results of Miyamoto and Auer were so surprising, there were a number of questions I wanted to answer for this Master's Thesis. Is intra-ischemic hyperoxemia really beneficial to stroke outcome? In addition to less brain damage, are there advantageous behavioral results as well? Is there a correlation between the degree of hyperoxemia and the amount of damage?

What happens when there are high levels of oxygen in the blood that *returns* to ischemic tissue? In other words, is the **reperfusion** period also a potential time for oxygen therapy, or would this cause more damage? What is the impact, both behaviorally and histologically, of combining intra-ischemic hyperoxemia with reperfusion hyperoxemia after a stroke?

It is conceivable that elevating blood oxygen levels could in fact be detrimental in combination with ischemia, due to widely accepted concepts of reperfusion injury and oxygen toxicity. It is important therefore to review these concepts before delving into the specifics of this thesis.

Reperfusion Injury

Oxygen-Derived Free Radicals

It is currently believed that a substantial amount of the damage resulting from an ischemic insult arises from the reintroduction of blood to the "dry" tissue. This theoretical necrosis secondary to that caused by the ischemia itself is called **reperfusion injury**, or post-ischemic injury[73]. This reperfusion injury is believed to be largely due to the production of oxygen-derived free radicals upon reintroduction of molecular oxygen to the ischemic tissue[73]. A free radical is a molecule containing an odd number of electrons, which gives it an "open" or "half" bond, thus rendering it extremely reactive[73]. Free radical production is believed to occur after ischemia because the decrease in blood flow is sufficient to limit oxygen availability needed to produce adequate amounts of ATP[73]. This drop in the amount of ATP in ischemic tissue begins a biochemical cascade which, upon reperfusion, produces large amounts of the superoxide radical and secondarily derived cytotoxic species capable of causing massive tissue damage[73].

Free radical scavengers are protective enzymes or drugs that efficiently and specifically react with the radicals, reducing their cytotoxicity[73]. Studies have examined whether attempting to reduce the amount of free radicals produced after a stroke by the administration of free radical scavengers could mitigate infarct size. Mizoi et al[74] found that administration of several different free radical scavengers with cerebral ischemia did

indeed reduce infarct size. These results have also been found by other researchers[75-81]. A study by Tan et al[82] found that fetal rabbits who were exposed to repetitive global ischemia-reperfusion exhibited greater cortical cell death than those rabbits exposed to sustained global ischemia-reperfusion and control animals. Maternal administration of free radical scavenging antioxidants resulted in less cortical and hippocampal cell death in the repetitive reperfusion group, which suggests that free radicals may be involved in global ischemia reperfusion. However, since free radical levels were not actually measured, any damaging affects of free radicals can not be confirmed. It has even been suggested that at least some of the neuroprotective effects of hypothermia with ischemia is due to a reduction in the amount of oxygen derived free radicals produced [83].

Hypoxic and Hyperoxic Reperfusion

Hypoxic reperfusion has been tested to examine whether reperfusion injury could be reduced by reducing the amount of oxygen in the blood returning to ischemic tissue. It was believed that reducing the substrate for oxygen-derived free radicals, namely oxygen, could reduce the damage. Ulatowski et al[84] found that hypoxic reperfusion did not improve brain electrical function after a period of global ischemia, and that the cerebral oxygen consumption did not differ between the normoxic and hypoxic groups. However, since free radical levels themselves were not tested, nothing can be confirmed about free radical involvement. Zwemer et al[85] found that hypoxic ventilation during cardiopulmonary resuscitation in dogs failed to improve neurological scores upon resuscitation although the necessary substrate for oxidant injury again was reduced. In this study free radical levels themselves unfortunately were not actually measured. These studies suggests that variations of reperfusion blood oxygen into the low range do not

affect ischemic damage, but since free radical levels were not measured, it remains unclear what role they play. As well, since these studies involved global ischemia, the role of hypoxic reperfusion in focal ischemia remains unclear.

Based on all of these theoretical considerations, high blood oxygen levels in the blood reintroduced to ischemic tissue might be damaging to brain tissue by augmenting the generation of oxygen-derived free radicals. It is not known conclusively whether such generation is harmful, or whether it is balanced by local improvements in tissue PO_2. A study by Lipinski et al[86] compared normoxic ventilation during resuscitation to hyperoxic ventilation after cardiac arrest, examining both neurobehavioral and histological outcomes. They found no difference between groups either behaviorally or histologically. Again, since no free radical measurement was performed, the role of oxygen-derived free radicals remains unclear. Agardh et al[87] examined the histological outcomes of hypoxic, normoxic, and hyperoxic reperfusion, and correlated this with free radical production. They concluded that there is no indication that variations in the post-ischemic oxygen supply altered the production of free radicals, or modulated the damage incurred as a result of ischemia. Together these studies suggest that variations of reperfusion blood into the high range after global ischemia does not influence infarct size.

The seemingly incompatible findings that free radical scavengers mitigate ischemic damage yet hyperoxemic reperfusion does not exacerbate ischemic damage can be understood if it is considered that any legitimate cytotoxic effects of free radicals may not be dose-dependent of the substrate. That is, free radical production and corresponding damage may be independent of arterial oxygen tension, existing as an "all-or-none" phenomenon. Research and treatment should approach free radicals and

reperfusion oxygen levels as separate entities.

Oxygen Toxicity

While it has not been proven that elevating blood oxygen levels in combination with ischemic tissue is pathological, hyperoxia has been shown to have some pathological effects which will now be briefly reviewed.

Hyperbaric Pressure

The initial work demonstrating oxygen toxicity was done at hyperbaric pressure. The concept of oxygen toxicity was pioneered by Paul Bert in 1878, who showed that oxygen could be highly poisonous and that no living matter was exempt[88]. Birds exposed to air at extremely high pressures, or **hyperbaria**, convulsed and ultimately died, and it was later deduced that oxygen was the key factor in these convulsions[88]. In 1899 Lorrain Smith found that mice that breathed oxygen at high barometric pressures (2.7-3.6 atmospheric pressures) developed fatal pulmonary damage after 5 to 10 hours[89]. Rats exposed to hyperbaric pressure have been shown to develop bilateral necrosis in selective regions of the CNS[90]. As early as 1910, experiments were being conducted on humans at hyperbaric pressure to examine the adverse effects of oxygen. In 1912 Bornstein suffered chronic leg spasms while riding an ergometer under 3 atmospheric pressures for 45 minutes. Behnke et al[91] found that breathing pure oxygen at 4 atmospheric pressures could produce convulsions in adult humans, but pulmonary symptoms were not produced. Blood pressure and respiratory rate were not affected in this study at hyperbaric pressures. Pulmonary distress, characterized by hyperventilation, substernal pain, and coughing, has been shown to by other investigators to occur in humans after 8 hours at 2 atmospheric pressures[89]. In 1947 Donald studied the effects of hyperbaric

oxygen in underwater divers. Divers were shown to be prone to lip twitching, nausea, auditory hallucinations, tinnitus and confusion, depending on depth and duration of exposure[88].

Normobaric Pressure

Oxygen toxicity exists not only at hyperbaric, but also at normobaric pressures. Mice have been shown to develop pulmonary damage from breathing pure oxygen at 0.8 atmospheric pressures (608 mm Hg) after 4 days[89]. Dogs have been shown to develop pulmonary damage from breathing pure oxygen at 1 atmospheric pressure after 12 to 18 hours[89]. Pulmonary distress has been shown to develop in humans as a result of breathing pure oxygen at normobaric pressure after 6 to 12 hours[89]. In the late 1960s and early 1970s it was further clarified that newborn bronchopulmonary dysplasia was due to oxygen toxicity[92]. A second example of the pathological effects of oxygen is a result of work done in the late 1940s and early 1950s, when it was recognized that retrolental fibroplasia in newborn infants was due to oxygen toxicity[92]. A third example of oxygen toxicity at normobaric pressure is seen in newborn babies, who, when exposed to prolonged elevation of blood oxygen levels, develop pontosubicular necrosis. This might be due to a selective decrease in cerebral blood flow with hyperoxia at this crucial time in development[93]. Similar neurotoxicity of oxygen has also been displayed in newborn rats, the toxicity decreasing with maturation[94].

Barometric Pressure and Arterial Oxygen Tension

In order to comprehend any potential infarct mitigating mechanisms of arterial hyperoxemia, the characteristics of gases, atmospheric oxygen, and oxygen delivery must be understood. **Boyle's general gas law** states that the pressure of a gas is inversely

proportional to its volume[95]. This is important because air flows from areas of higher to lower pressure. Inspiration results when alveolar volume increases, causing intrapulmonary pressure to decrease below atmospheric pressure[96]. **Dalton's gas law** states that in a mixtures of gases, each gas behaves as if it alone occupied the total volume and exerts a pressure (partial pressure) independently of the other gases present[95].

Alveolar Gases at Sea Level

At sea level, the total barometric pressure (760 mm Hg) consists of a partial pressure of nitrogen (PN_2; 600.2 mm Hg, 78.98%), and a partial pressure of oxygen (PO_2; 159.5 mm Hg, 20.98%)[96]. Carbon dioxide accounts for 0.3 mm Hg (.04%) of this atmospheric air. After this dry atmospheric air is inhaled into the lungs and carried toward the alveolar spaces, it becomes saturated with water, which evaporates from the surface of the tissue. The water vapor is similar to other gases in that it exerts a partial pressure and behaves independently from the other gases in the mixture. However, since it is in equilibrium with its liquid phase, water vapor's partial pressure depends almost completely on temperature and is almost independent of the barometric pressure[97]. At normal body temperature the PH_2O is 47 mm Hg[96]. Accounting for this uniform presence of water vapor in alveolar air, the total pressure of the other gases is equal to the barometric pressure minus 47 mm Hg[97]. Therefore, to calculate the partial pressure of an alveolar gas, one multiplies its fractional concentration by the barometric pressure minus 47.

$$P_A = F_A \times (\text{barometric pressure} - 47)$$ [97]

Because the concentration of nitrogen in alveolar air is approximately 80%, that of oxygen is approximately 14%, and that of carbon dioxide is approximately 6%[97], their

partial pressures at sea level are approximately:

$PN_2 = .80 \times (760 \text{ mm Hg} - 47 \text{ mm Hg}) = 570 \text{ mm Hg}$

$PO_2 = .14 \times (760 \text{ mm Hg} - 47 \text{ mm Hg}) = 100 \text{ mm Hg}$

$PCO_2 = .06 \times (760 \text{ mm Hg} - 47\text{mm Hg}) = 43 \text{ mm Hg}$ (table 1)

	SEA LEVEL				CALGARY			
	Dry Air		Alveolar Air		Dry Air		Alveolar Air	
	Mm Hg	%	Mm Hg	%	Mm Hg	%	Mm Hg	%
PN_2	600.2	78.98	570	75.0	526.6	78.975	496	74.4
O_2	159.5	20.98	100	13.2	139.9	20.98	87	13.0
PCO_2	0.3	0.04	43	5.6	0.3	0.045	37	5.5
PH_2O	--	--	47	6.2	--	--	47	7.0
TOTAL	760	100	760	100	667	100	667	100

Table 1 - Partial pressures of gases at sea level and in Calgary.

Henry's gas law states that the amount of gas dissolved in a liquid with which it is in contact is directly proportional to the partial pressure of the gas[95]. Deoxygenated blood flowing into the alveolar capillary from the rest of the body has a PO_2 of approximately 40 mm Hg[96]. Consequently, oxygen diffuses from the alveolar space down its concentration gradient across the respiratory membrane into the alveolar capillary. By the time the blood flows through the first third of the pulmonary capillary, an equilibrium is achieved and the PO_2 in the blood is approximately 100 mm Hg, equivalent to the PO_2 in the alveolar space [96]. This oxygenated blood then mixes with shunted (deoxygenated) blood from the alveoli, bronchi, and bronchioles. As a consequence, blood leaving the lungs through the pulmonary veins and returning to the heart has a PO_2 of approximately **95 mm Hg**[96] at sea level.

Alveolar Gases in Calgary

Due to its altitude, the atmospheric pressure in Calgary, 667 mm Hg, is considerably less than that at sea level. The PO_2 of pulmonary blood differs accordingly. Because the absolute amount of CO_2 in Calgary's atmospheric air remains the same as at sea level (0.3 mm Hg, .045%), due to a uniform amount produced through the respiration of animals, the percentages of nitrogen and oxygen in Calgary's air differ slightly from that at sea level. The total atmospheric pressure in Calgary therefore consists of a PN_2 of 526.6 mm Hg (78.975%) and a PO_2 of 139.9 mm Hg (20.98%). Following the inhalation of this dry atmospheric air into the alveolar space, the partial pressures of nitrogen, oxygen, and carbon dioxide in Calgary become approximately:

$PN_2 = .80 \times (667 \text{ mm Hg} - 47 \text{ mm Hg}) = 496 \text{ mm Hg}$

$PO_2 = .14 \times (667 \text{ mm Hg} - 47 \text{ mm Hg}) = 87 \text{ mm Hg}$

$PCO_2 = .06 \times (667 \text{ mm Hg} - 47 \text{ mm Hg}) = 37.2 \text{ mm Hg}$ (table 1)

Accounting for the mixture of shunted blood, the normal PO_2 of blood returning to the heart in Calgary is approximately **85 mm Hg.**

Production of Arterial Hyperoxemia

By breathing in pure oxygen, and therefore eliminating the partial pressure of the predominantly nitrogen in alveolar air, the PO_2 of alveolar air at sea level can be raised to:

100% (PO_2)

-5.6% (PCO_2) 88.2 % × 760 mm Hg = 670.3 mm Hg

-6.2% (PH_2O)

88.2%

Following the same logic, the PO_2 of alveolar air in Calgary can be raised to a theoretical maximum of:

100 % (PO_2)

-5.5 % (PCO_2) 87.5 % × 667 mm Hg = **583.6 mm Hg**

-7.0 % (PH_2O)

87.5%

While it is theoretically possible to raise the PO_2 of pulmonary capillary blood to this elevated level, pilot studies showed that this is not so easy. One reason for this is that the lining of the alveolar spaces, the interstitial spaces, and the walls of the lung capillaries, together called the **respiratory membrane**, are barriers to the diffusion of oxygen from alveolar air to the blood. This makes a complete equilibrium between alveolar air and blood impossible[95]. Another possible reason is that tracheal intubation, which is necessary in this experiment for the delivery of the inhaled anesthetic halothane and the maintenance of adequate ventilation (see below), may cause a slight fluid build-up and **edema** of the lungs. This may effectively thicken the respiratory membrane and slow the diffusion of oxygen across the membrane into the pulmonary capillaries. A third possible reason that the blood oxygen levels do not reach the theoretical maximum could be due to a **ventilation/perfusion mismatch**. The gas composition of the blood leaving the lungs is determined by the ratio of alveolar ventilation to the pulmonary capillary blood flow[97]. Although increasing the oxygen concentration in the air of the lungs will cause the relaxation of precapillary sphincters and increased blood flow in the lungs[96], this increase in pulmonary blood flow may not be enough to maximize blood oxygen levels. As well, since animals must be in the supine position during their surgery,

this unnatural position may cause mechanical compression of the lungs, resulting in decreased pulmonary perfusion. Finally, according to **Henry's Gas Law**, the concentration of a gas dissolved in a liquid is equal to the partial pressure of the gas over the liquid times the solubility coefficient of the gas. Therefore, a limited amount of oxygen, with a solubility coefficient of 0.023, can be dissolved across the respiratory membrane[96].

Oxygen Transport in the Blood

Hemoglobin

We next turn our attention to how oxygen travels in the blood, and the effect of arterial PO_2 (PaO_2). **Hemoglobin** is a red protein of erythrocytes that consists of 4 globin chains and 4 heme groups, each containing an iron atom. Because of the additional binding sites for oxygen on the iron atom, hemoglobin greatly increases the ability of blood to carry oxygen[20]. Most arterial oxygen is carried through the blood bound to hemoglobin, with a minority of oxygen dissolved freely in the blood serum. For example, at a sea level normoxemic PaO_2 of 100 mm Hg, a total of 19.80 ml of oxygen are present in every 100 ml of blood (~20%)[98]. Of this 19.80 ml of oxygen, 19.50 ml are bound to hemoglobin, and the remaining 0.30 ml of the oxygen are dissolved freely in the blood serum[98]. In other words, at a PaO_2 of 100 mm Hg, 98.5% of the oxygen in the blood is bound to hemoglobin, and the remaining percentage is dissolved freely in the blood.

Oxygen-Dissociation Curve of Hemoglobin

At a PaO_2 of 100 mm Hg, 97.5% of the possible hemoglobin binding sites (4 per hemoglobin protein) are saturated with oxygen[98]. The PO_2 greatly influences the degree

of hemoglobin-oxygen saturation. At a PO_2 of 40 mm Hg, which is approximately equal to the PO_2 of the blood in veins, a total of 15.12 ml of oxygen are present in every 100 ml of blood. Of this total amount of oxygen, 15.00 ml are bound to hemoglobin, and the remaining 0.12 ml of oxygen are dissolved freely in the blood[98]. This translates into 99.2% of the oxygen in this blood bound to hemoglobin. However, at this PO_2, only 75% of the possible hemoglobin binding sites are saturated with oxygen[98].

The relationship between PO_2 and hemoglobin saturation is described by the oxygen-dissociation curve of hemoglobin (figure 2)[99]. This curve is distinctly sigmoidal, with the steepest part of its slope occurring at the levels of oxygen tension corresponding to those found in tissue[99].

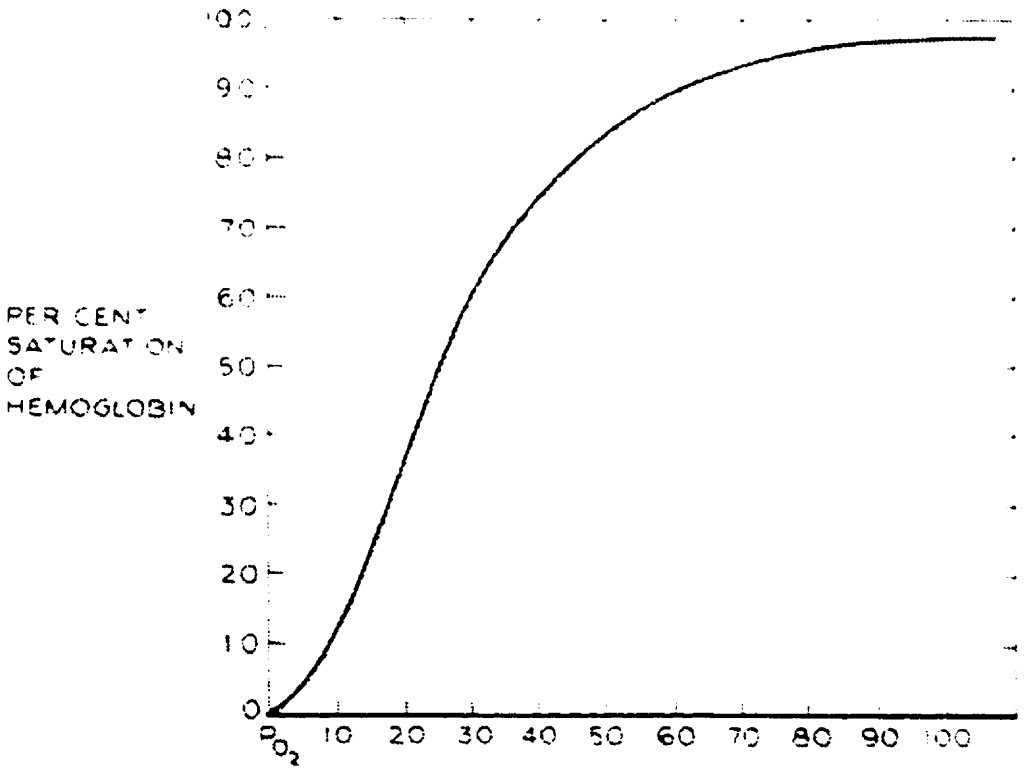

Figure 2 - Oxygen-dissociation curve of hemoglobin. From Ranney and Sharma, 1995.

Oxygen Affinity to Hemoglobin

Oxygen affinity to hemoglobin, or the "stickiness" of oxygen to hemoglobin, can be influenced by pH, the concentration of 2,3-BPG, and temperature[99], and can affect how much oxygen actually gets from the blood to the desired tissue. Changes in these parameters cause the oxygen-dissociation curve of hemoglobin to shift either left or right. For example, the **Bohr** effect describes the reduction in oxygen affinity and corresponding shift of the oxygen-dissociation curve of hemoglobin to the right as a result of a reduction of blood pH[99]. With this phenomenon, when blood reaches the tissue where the PO_2 is lower and the concentration of hydrogen ions is increased by lactic acid or CO_2 from cellular respiration (thus lowering blood pH), the Bohr effect reduces the affinity of oxygen to hemoglobin (*i.e.* makes it less "sticky"). This facilitates the transfer of oxygen from the blood to the tissue[98].

Another factor that affects oxygen affinity is the concentration of 2,3-bis (phosphoglyceric acid) (2,3-BPG). This substance is an intermediate product of glycolysis that is present within red blood cells at a concentration equimolar to hemoglobin[100]. Anemia (a reduction in the amount of red blood cells or hemoglobin), hypoxia, and the ascent to high altitudes all produce increased concentrations of 2,3-BPG[98]. Increases in 2,3-BPG concentrations progressively decrease oxygen's affinity to hemoglobin, shifting the oxygen-dissociation curve to the right. This consequently eases the delivery of oxygen to the tissue[100]. A third factor which influences the affinity of oxygen to hemoglobin is temperature. Lower temperatures are known to increase oxygen affinity, while increased temperatures decrease oxygen affinity[98].

Hemoglobin-Bound vs. Freely Dissolved Oxygen

As mentioned above, according to the oxygen-dissociation curve of hemoglobin, when the PO_2 of blood reaches 100 mm Hg, 97.5% of the hemoglobin binding sites are saturated with oxygen. Progressively increasing the oxygen tension in the blood from the normoxemic to the hyperoxemic range produces the complete saturation of hemoglobin, beyond which oxygen enters the bloodstream only as physically dissolved in the blood serum[101]. This concept is a very important one for this thesis. The solubility of oxygen in the blood serum is not only a function of the PO_2 in the alveolar space, but also of the solubility coefficient of oxygen in serum[101]. The volume of freely dissolved oxygen in the blood with a PaO_2 of 100 mm Hg at sea level can be obtained from the following equation:

$$100 / 760 \times 2.3\% \text{ volume} = 0.30\% \text{ volume}$$

where 2.3% volume represents the solubility coefficient of oxygen for each atmosphere of oxygen[95,101]. The volume of freely dissolved oxygen in the serum at sea level can be theoretically raised, after increasing the fraction of inspired oxygen to 100%, to:

$$(760 - 43(PCO_2) - 47(PH_2O))/760 \times 2.3\% \text{ volume} = 2.0\% \text{ volume}$$

In Calgary, with its reduced atmospheric pressure, the amount of freely dissolved oxygen in the blood at a PO_2 of 100 mm Hg would equal:

$$100 / 667 \times 2.3\% \text{ volume} = 0.34\% \text{ volume}$$

Analogously, in Calgary, the volumes of freely dissolved oxygen in the blood serum at PaO_2s of 50 mm Hg, 200 mm Hg, 300 mm Hg, and 400 mm Hg would equal:

$$50 / 667 \times 2.3\% \text{ volume} = 0.17\% \text{ volume}$$

$$200 / 667 \times 2.3\% \text{ volume} = 0.69\% \text{ volume}$$

$$300 / 667 \times 2.3\% \text{ volume} = 1.03\% \text{ volume}$$

$$400 / 667 \times 2.3\% \text{ volume} = 1.38\% \text{ volume}$$

respectively.

Oxygen Delivery to Tissue

After oxygenated blood has left the pulmonary capillaries, it travels into the pulmonary veins and into the left atrium and ventricle of the heart. From there it is pumped into the aorta and subsequently into the arteries of the body. When the arteries reach an organ, the vessels narrow until they become capillaries. Substances, such as oxygen, can cross the capillary wall by diffusing through endothelial cells, through fenestrae, and between the endothelial cells. Oxygen is lipid soluble and therefore can easily diffuse through the cell membrane down the concentration gradient to the oxygen-poor tissue. Due to the Bohr effect (see above), hemoglobin's affinity to oxygen is reduced and oxygen can be more readily obtained by the tissue.

The Middle Cerebral Artery

Our attention will now be shifted from blood oxygenation to one of the major arteries of the brain. This artery is a common site for blockage in humans, and is therefore often the site for occlusion in stroke research.

Subcortical Vascularization

In humans, the large **middle cerebral artery (MCA)** bifurcates from the internal carotid artery and proceeds into the lateral sulcus[1]. As it proceeds towards and through this sulcus, it gives rise to several branches that penetrate and supply blood to deep structures of the brain: the diencephalon, the basal ganglia, and the internal capsule (figure 3). These branches are known as the **lenticulostriate** branches of the MCA[102].

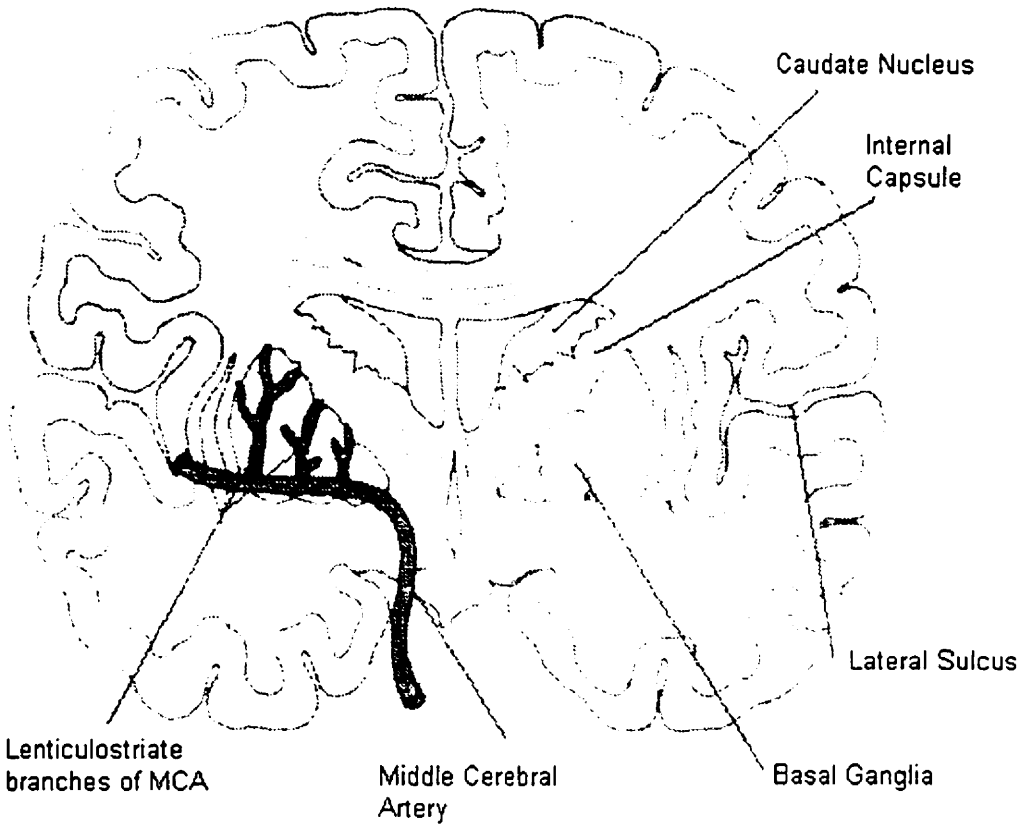

Figure 3 - Coronal view of subcortical vascularization of the middle cerebral artery in humans. Adapted from Diamond et al, 1985.

The branches are often referred to as end-arteries since there is inadequate collateral circulation within their areas of circulation[102]. For this reason, obstruction of these small vessels brings on serious neurological deficit, and the deficit is often out of proportion to the size of infarct. For example, somatosensory projections from the thalamus pass through the internal capsule on their way to the postcentral gyrus. Infarction of a small part of the internal capsule can cause neurological deficits similar to those resulting from a massive infarct of the cortex[1]. Due to the inadequate collateral circulation, infarction of the deep structures of the brain is hard to treat clinically.

Cortical Vascularization

On its course through the lateral sulcus, the MCA divides into a number of

branches that supply the insula[1]. The MCA emerges from the lateral sulcus to supply almost the entire surface of the cerebral hemisphere, or the cortex, with its distal branches[1](figure 4). Occlusion of the MCA in its distal regions, if there is not

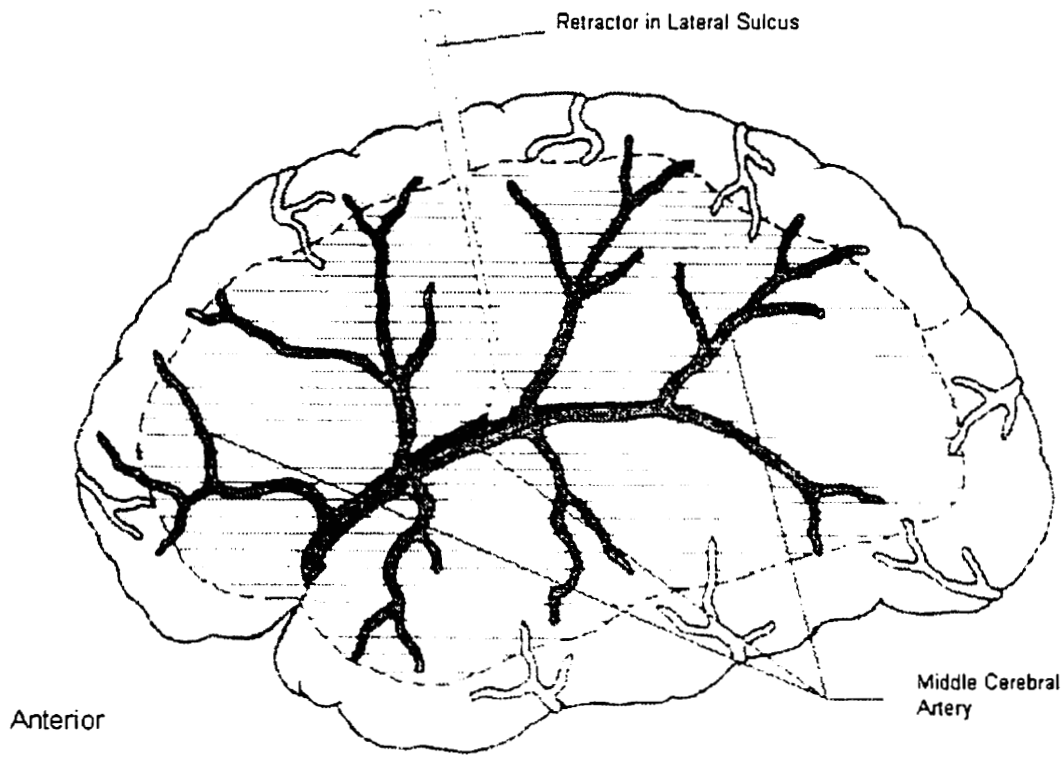

Figure 4 - Lateral view of cortical vascularization of the middle cerebral artery in humans. Adapted from Diamond et al, 1985.

compensatory blood flow from collateral arteries, may deprive such functionally important cortical areas such as the somatosensory cortex, motor cortex, auditory cortex, and, if the left side is involved, speech centres[1,102].

The Middle Cerebral Artery of the Rat

The structure and function of the middle cerebral artery in human and rats appears to have been preserved through evolution. In general, the anatomic distribution of the MCA in rats is analogous to that in humans, and the neural structures supplied are

comparable between species[103,104]. For this reason, we infer that stroke research in this species can be used to study stroke phenomena in humans.

Stroke Model

We will now focus our attention on the experiments undertaken for this thesis on hyperoxemia and focal cerebral ischemia. The stroke model used in this thesis was to occlude the middle cerebral artery with an intra-luminal suture in rats, first described by Koizumi et al[105] and Longa et al[103]. The intention of using animals for medical research is to mimic as closely as possible the condition seen in humans. This model was chosen for a few reasons. First, to occlude an artery from the inside, as opposed to clipping an artery from the outside, is more representative of human stroke. With human stroke there is no craniectomy, or hole drilled into the skull, there is no disruption in the integrity of the meninges, and there is no arterial crush injury associated with an external clip. The external clip model is a good animal replication of iatrogenic stroke, caused by neurosurgeons who may clip an artery while repairing an aneurysm. Second, the middle cerebral artery in both rats and humans is a longitudinal continuation of the internal carotid artery, and therefore is a common site for strokes due to embolism from another part of the body. Indeed, the middle cerebral artery is often termed the "artery of embolus". Third, to occlude one artery as opposed to disrupting the entire blood supply to the brain is more representative of stroke, whereas the latter is more representative of cardiac arrest.

Behavioral and Histological Assessment

It is equally as important to assess neurobehavioral deficit as it is to quantify infarct size when considering therapies for ischemic injury. Neurobehavioral deficit is,

after all, the proximate cause of disability in stroke, and is mediated by integrity of the neurons. There are two possible ways that ischemia could affect behavior – by altering the synapses of neurons, and therefore neurotransmission, and by destroying areas of the brain involved with the behavior. Reducing the amount of necrosis will benefit the behavior of the individual if functionally important areas of the brain are preserved. Since patients presenting with stroke symptoms are concerned more with alleviating the symptoms than reducing the size of damage, discernable only with neuroimaging and upon autopsy, the ultimate goal of stroke research is to improve the lives of those who have suffered this injury. Reducing the amount of tissue death in the brain is a powerful way to reduce behavioral deficit after a stroke.

While neurological deficit has been shown to correlate significantly with the size of the infarcted area[106], there are at least four conceivable reasons that the two measurements would not correlate. If ischemic damage was limited to a change in either the presynaptic or postsynaptic terminal, while neurons were not killed, there could be an alteration in behavior without corresponding necrosis. As well, if the damage involves a major tract of white matter involved in motor control, a small infarct may have large behavioral outcome, before the anterograde degeneration of the neuron has a chance to increase infarct size[107]. A third reason behavior may not correlate with histology is that animals, as well as humans, who have identical histology still exhibit variations in behavior. A fourth possible reason that behavior and histology would not correlate after an ischemic insult is that behavior improves over time as the brain "re-wires" itself and the individual learns how to cope with the injury. The amount of tissue lost in the brain, however, increases over time as necrotic tissue is cleaned up, and transynaptic atrophy

occurs. The timing of the neurological testing, and the length of the survival period, therefore, would influence whether behavior and histology correlate.

Arterial Oxygen Variation in this Thesis

To examine the relationship between arterial hyperoxemia and ischemia, the temporal acquisition of elevated blood oxygen levels was varied to during (intra-ischemic hyperoxemia), after (reperfusion hyperoxemia), and both during and after (intra-ischemic plus reperfusion hyperoxemia) a rat had a stroke. Differences between groups were judged by comparing both the behavior after the stroke, and the size of infarcts. The hyperoxemic groups were compared to a normoxic control group, who experienced normal blood oxygen levels during and after their strokes.

In the first experimental condition, it was hypothesized that those rats who were exposed to intra-ischemic hyperoxemia would exhibit improved neurobehavioral scores and smaller infarcts than control rats, and further that their infarct size would decrease as the PO_2 increases. Since ischemia, by definition, does not allow the direct delivery of oxygen to tissue through the blood, a question arises as to how intra-ischemic hyperoxemia may reduce infarct size, as reported by Miyamoto and Auer[70]. It is proposed here, although not directly tested, that the elevated amount of freely dissolved oxygen in the blood serum may diffuse across the concentration gradient from vascularized areas to the ischemic tissue, thereby maintaining ATP levels in ischemic tissue, and the reducing the free radical production upon reperfusion. By reducing this free radical production, the reperfusion injury can be minimized, thus salvaging much, if not most, of the ischemic tissue. Increasing PO_2 levels will allow the diffusion of more oxygen into the ischemic tissue, hypothetically producing a correlation between blood

oxygen level and infarct size.

In the second experimental condition, it was hypothesized that those rats who are exposed to reperfusion hyperoxemia will exhibit impaired neurological scores and larger infarcts than control rats, due to a larger free radical substrate in the blood and a larger corresponding production of theoretically necrotizing free radicals. The reperfusion hyperoxemia condition was added to this study to critically test the relationship of reperfusion injury to blood oxygen level. While free radical levels themselves were not measured in this thesis, any detrimental effects seen with hyperoxemia could be dissected out and classified as being due either to hyperoxemia itself or hyperoxemia-mediated reperfusion injury.

In the third experimental condition, it was hypothesized that those rats who experienced hyperoxemia both during and after their stroke would exhibit improved neurological scores and smaller infarcts than control rats, similar to the intra-ischemic hyperoxemia groups. This condition was added to the study to maximize clinical applicability, envisioning the possibility of oxygen therapy for stroke patients during and after their ischemia. Clinically, it is impossible to determine exactly when the moment of reperfusion occurs. Again, because of the proposed neuroprotective effect of intra-ischemic hyperoxemia diffusing from vascularized areas to ischemic tissue, it was believed that those rats would not suffer a critical drop in the levels of ATP, and would therefore experience a lesser degree of free radical reperfusion injury. Hyperoxic reperfusion after hyperoxic ischemia was hypothesized to restore oxygen tension to the tissue rather than produce tissue damaging free radicals.

What the results will tell us

The results of the aforementioned experiments will tell us whether elevating blood oxygen levels during and after a stroke is beneficial, at least in the species utilized for this experiment. However, there are reasons to believe that any results showing beneficial effects of hyperoxia in rodents may not be directly applicable to humans. There are fundamental neurological differences between the species which are important to consider when interpreting results. As brain size increases throughout the animal kingdom, there is a corresponding decrease in the neuronal density. That is, larger brains have larger spaces between neurons. Since neuronal density determines cerebral metabolic rate, and cerebral metabolic rate determines cerebral blood flow, there are relevant differences in these parameters between rats and humans. Besides the neuronal differences between species are vascular differences and oxygen diffusion differences: The middle cerebral artery of the rat is the size of only a small branch of the human MCA, and oxygen must diffuse much farther to reach ischemic tissue in the larger, human brain.

Therapies which work by manipulating cerebral metabolism, cerebral blood flow, or oxygen diffusion may be species specific, as the pre-existing differences in the basal rates of these parameters between species could be different enough to prohibit therapies from working across the animal kingdom.

CHAPTER TWO: METHODOLOGY

Subjects and Groups

Fifty-two male Wistar rats (350-400g) were divided into 5 groups, namely the normoxemic/control (Norm; n=10), low intra-ischemic hyperoxemia (lo-Isch; n=10), high intra-ischemic hyperoxemia (hi-Isch; n=11), reperfusion hyperoxemia (Reper; n=10), and intra-ischemic plus reperfusion hyperoxemia (I + R; n=11) groups. The groups were filled concurrently to eliminate any cumulative practice effect of the experimenter on the surgery. Because it was impossible to predict the degree of hyperoxemia that would be reached in the intra-ischemic hyperoxemia groups, animals were assigned to these groups after all surgeries were completed based on their average blood oxygen level during their stroke. Rats had access to food and water *ad libitum* before and after the surgery.

Pilot Studies

The duration of ischemia and the intra-ischemic blood pressure were varied in pilot studies in an effort to produce consistent infarcts with low mortality. It was found that a stroke of long duration (90-120 minutes) with normal blood pressure (80 mm Hg) produced consistent subcortical damage, but very inconsistent cortical damage, likely due to collateral blood flow supplying the tissue. Strokes of shorter duration (30 minutes) and very low blood pressure (40-50 mm Hg) produced consistent cortical and subcortical infarction, yet animals often developed fatal seizures. It was determined that a 60 minute stroke at a blood pressure of 60 mm Hg produced the most consistent cortical and subcortical damage with very low mortality. These were therefore the parameters used in this thesis.

Pre-Stroke Preparation

Rats were anesthetized by inspiration of 3.5% halothane in an 80% nitrous oxide and 20% oxygen gas mixture. Tracheal intubation was done by guiding a 5 cm long polyethylene tube having an inner diameter of 1.67 mm and an outer diameter of 2.42 mm (PE 240 tubing, Clay Adams, Parsipanny, NJ) between the vocal cords. The animals were subsequently maintained on a Starling-type ventilator with a 1-1.5% halothane, 80% nitrous oxide, 20% oxygen mixture. The tail artery was then isolated and cannulated using polyethylene tubing with an inner diameter of 0.58 mm and an outer diameter of 0.965 mm (PE 50 tubing, Clay Adams, Parsipanny, NJ). This arterial line was connected to a Statham transducer, and the blood pressure was automatically displayed on a computer monitor every second. The blood pressure was recorded by the computer every 5 seconds. Blood samples were also obtained through this arterial line. Blood gases and pH were analyzed using a 1304 pH/blood gas analyzer (Instrumentation Laboratories, Milan, Italy) before, during, and after ischemia. Blood glucose was measured using a "One Touch II" blood glucose meter (Lifescan Canada, Ltd.) before, during, and after ischemia. The hematocrit, or percentage of red blood cell volume in serum, was obtained from a Readocrit centrifuge (Clay Adams, N.J) before, during, and after ischemia.

Body temperature was controlled to the physiological level (37.0 ± .2°C) using an overhead lamp and a thermistor-regulated servocontrolled heating blanket operating on a feedback system with the core temperature, obtained by a 6 cm rectal temperature probe. The brain temperature of rats in this experiment, as inferred by ipsilateral middle ear temperature, was strictly controlled to a normothermic level (37.0 ± .2°C) during the

ischemic and monitored reperfusion period. This was accomplished by re-establishing normothermia with the overhead lamp before MCA occlusion (brain temperature tends to drop when neck vessels are exposed to ambient air), and covering the animal's head in a scrub blanket. Preliminary studies showed it was quite easy to maintain normothermia with this protocol.

Middle Cerebral Artery Occlusion

All rats experienced middle cerebral artery occlusion with an intraluminal thread (figure 5). After a right ventrolateral neck incision, the sternomastoid, digastric, and omohyoid muscles were isolated and separated, exposing the right **common carotid artery**. The occipital and superior thyroid branches of the **external carotid artery (ECA)** were then isolated and coagulated. The ECA was tied off using a 6-0 silk suture, and cut with a micropolar cautery apparatus (model SSE2L, Valleylab, Boulder, Colorado). A microvascular clip was then temporarily placed at the origin of the ECA, and a 6-0 silk suture was loosely tied around the ECA stump. The ECA stump was then cut with fine surgical scissors, and a 21mm length of 3-0 monofilamont nylon suture was introduced into the stump, eventually to be advanced into the **internal carotid artery (ICA)** to block the origin of the MCA. The silk suture was tightened around both the ECA stump and the intraluminal suture to prevent bleeding, and the clip was removed. The nylon suture was then advanced into the internal carotid artery exactly 21 mm from the point where the common carotid artery bifurcates into the ECA and ICA. At the moment the nylon suture was advanced 21 mm into the ICA, the period of ischemia began, as the suture occluded the origin of the middle cerebral artery. In an effort to reduce collateral blood flow from the Circle of Willis, blood pressure was dropped to 60

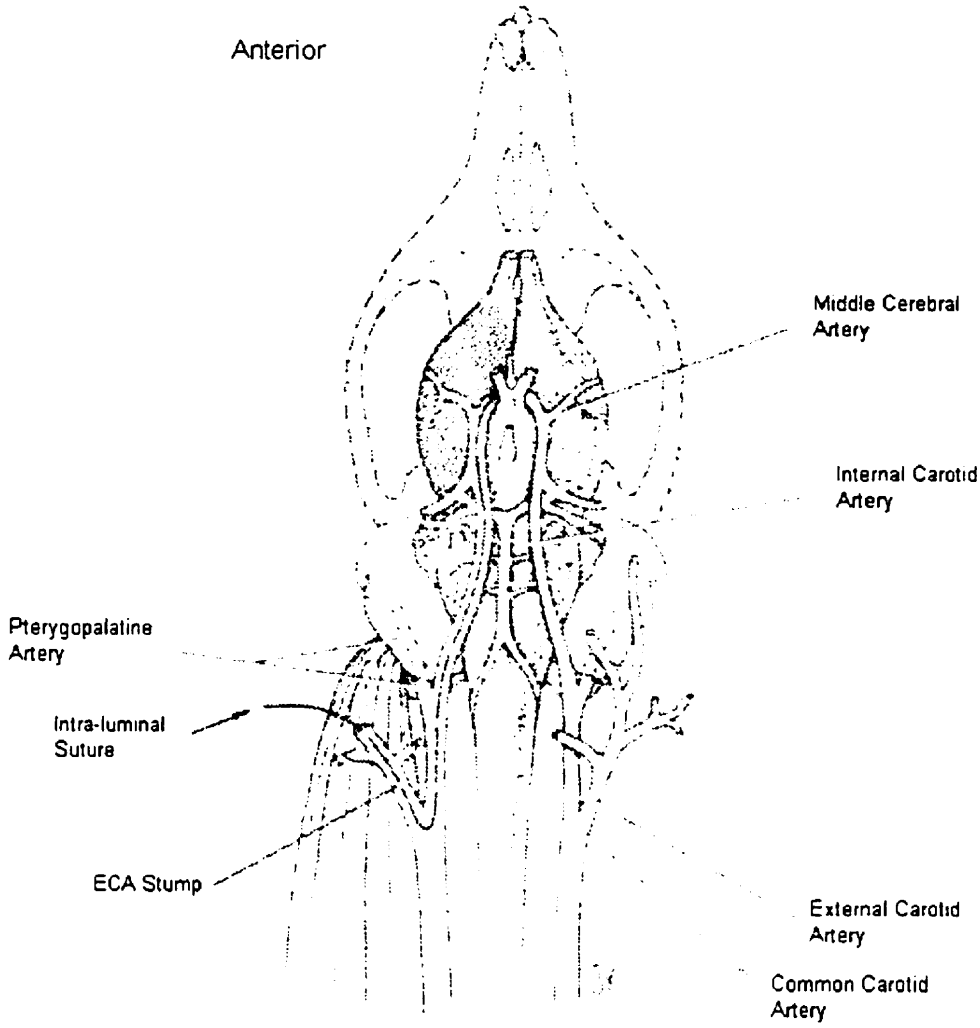

Figure 5 - Cerebral vascularization of the rat brain. Note the suture within the internal carotid artery, occluding the origin of the middle cerebral artery. Adapted from Longa et al, 1989.

mm Hg by immediately increasing the percentage of halothane inspired (~2.0%) at the beginning of the ischemic period. Because ischemic blood pressure has been shown to greatly influence infarct size[61], the blood pressure was strictly controlled (± 4 mm Hg) to the designated level.

Variations in Oxygen Inspiration

For the intra-ischemic hyperoxemia groups (lo-Isch, hi-Isch, I+R), the fraction of inspired oxygen was increased to 100% at the same time the level of halothane was

increased (*i.e.* the very beginning of ischemia). For the other groups (Norm, Reper), the fraction of inspired oxygen remained the same throughout the ischemic period (*i.e.* yielding normoxemia).

After the 60 minute MCA occlusion, the nylon suture was then withdrawn and the ECA stump was tied permanently. For the reperfusion hyperoxemia group, the fraction of inspired oxygen was increased 59 minutes into ischemia, allowing 1 minute for hyperoxemia to be established before revascularization. The intra-ischemic plus reperfusion hyperoxemia group maintained elevated blood oxygen levels throughout the ischemia and reperfusion periods. The fraction of inspired oxygen was dropped back down to the pre-ischemic levels (normoxemia) for the intra-ischemic groups (lo-Isch, hi-Isch) 59 minutes into ischemia, allowing the re-establishment of normoxemia before revascularization. The control group experienced no increase in the fraction of oxygen inspired (table 2).

Group	Ischemia	Reperfusion
Normoxemia	N	N
Low Ischemic Hyperoxemia	↑	N
High Ischemic Hyperoxemia	↑↑	N
Reperfusion Hyperoxemia	N	↑
Ischemia plus Reperfusion Hyperoxemia	↑	↑

Table 2 - Variations of oxygen inspiration in the present experiment (N = normoxia, ↑ = hyperoxia).

At the beginning of the reperfusion period, blood pressure was returned to normotensive levels by reducing the concentration of halothane inspired to 1%. The rats then experienced a monitored 60 minute reperfusion period, during which time the neck

wounds were closed. Halothane was discontinued 55 minutes into the perfusion period. As well, the last blood sample was taken at this time, allowing 5 minutes to close the tail wounds before the ventilator was turned off at exactly 60 minutes after the moment of reperfusion. The animals were then extubated and allowed to awaken.

In an effort to maximize clinical applicability, the reperfusion hyperoxemia groups (Reper and I + R) spent the first 24 hours post-surgery in an oxygen rich environment, mimicking oxygen therapy for those individuals who have suffered a stroke. This was accomplished by placing rats in a 50 cm × 30 cm × 30 cm closed anesthesia box, with a humidified 100% oxygen input. At the end of this 24 hour period, rats were returned to cages and exposed to normoxic ambient air. The normoxemic and intra-ischemic hyperoxemia groups also spent the first 24 hours post-operative in this anesthesia box, but were exposed to ambient air rather than an oxygen rich environment.

Bederson Neurological Exam

24 hours after surgery, rats were given the Bederson Neurological Exam[106], with scores ranging from 0-3. Rats with no observable impairment scored a 0. Rats were held gently by the tail, and observed for forelimb flexion. Normal rats will extend both arms toward the floor in an effort to break their fall – rats who displayed consistent forelimb flexion contralateral to the injured hemisphere scored a 1. Rats were then lowered and allowed to grasp the bars on the top of their cage. Animals who displayed consistent contralateral limb weakness scored a 2. Rats were then returned to their cage and observed. Rats who circled toward the paretic side scored a 3.

Survival Period

Rats in all groups were allowed a 14 day survival period. This period of survival was chosen because pilot studies showed that by this time all edema of the brain has regularly dissipated, easing calculation of atrophy (see below). Any animal that died before 14 days was excluded from the behavioral and histopathological analysis and used only for mortality calculation. During the 14 day survival period, the amount of water consumed and any change in body mass were recorded.

Histological Preparation

Transcardiac perfusion was performed at 14 days survival under 2% halothane with saline at a flow rate of 130 ml/min for 4 minutes (approx. 500 ml) to wash blood from the tissue, followed by 4% formaldehyde fixative at a flow rate of 11 ml/min for 20 minutes. Rats were then decapitated and the heads were allowed to soak in 4% formaldehyde overnight. Brains were removed after 12 hours and cut coronally into 4 mm sections.

The brain tissue was then processed with a series of graded alcohols (dehydration), xylene (dealcoholization), and paraffin wax (wax impregnation). The tissue was then embedded into paraffin wax blocks (Tissue-Tek).

A total of 13 coronal sections, ranging from the olfactory bulbs to the occipital cortex (stereotaxic levels 14.2 - 2.2)[108] were obtained for each rat. Sections were cut with a sliding microtome (820 Histocut, Leica, Germany) 8 μm thick, 1 mm apart. Sections were then floated in a water bath to rehydrate the tissue, and caught on a microscope slide (Surgipath Precleaned Microslides, Winnipeg, Manitoba). After the sections were

sufficiently dried and fixed to the microscope slide with a heating lamp, they were then stained with hematoxylin and eosin acid/base stains (Surgipath, Winnipeg, Manitoba).

Infarct Quantification

The infarct size for each rat, considered to be the areas of pan-necrosis, was then calculated. Because the band of selective neuronal necrosis surrounding the area of pan-necrosis (the penumbra) in this experiment was minimal, often non-existent, any selective neuronal necrosis observed was not classified as part of the infarct in this experiment to ensure the consistency of infarct quantification. On each of the coronal sections, a total of 4 polygons (figure 6) were traced using a video analysis system (Image Pro Plus, Media Cybernetics, Maryland), under operator control. Two polygons were traced within the affected hemisphere, one for the cerebral **cortical necrosis** and the other for **subcortical necrosis**. Ischemic infarcts are easily identifiable and can be defined as containing 1) cytoarchitectural (including neuropil) disruption, 2) hypereosinophilia

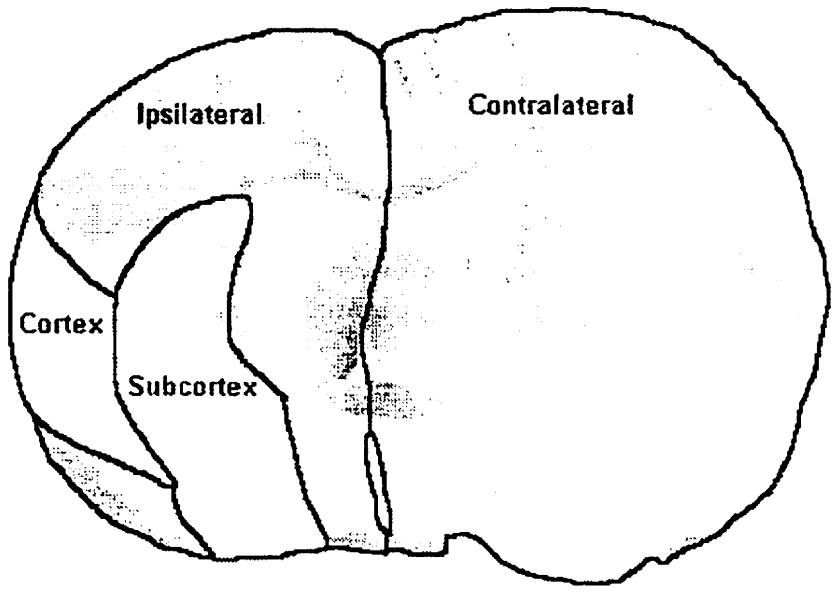

Figure 6 - Example of a coronal section of a rat; with cortex, subcortex, ipsilateral, and contralateral polygons shown.

(affinity for acid dyes, giving rise to excessive pink staining), and/or 3) dense sheets of microglia. Controlling for the exact demarcation of the infarct border was done using a light microscope in conjunction with the video analysis system. Because of **atrophy** in the ischemic hemisphere, due to the removal of necrotic tissue by macrophages and trans-synaptic degeneration, two additional polygons were measured, comprising the entire areas of both the ischemic (**ipsilateral**) and unaffected (**contralateral**) hemispheres (figure 6).

The total amount of necrosis per section was obtained by adding the cortical necrosis to the subcortical necrosis:

Total Necrosis = Cortical Necrosis + Subcortical Necrosis

The amount of atrophy per section was calculated by subtracting the total area of the ischemic hemisphere, including the areas of necrosis, from the total area of the contralateral hemisphere:

Atrophy = Contralateral Hemisphere – Ipsilateral Hemisphere

The total tissue loss per section was then obtained by summing the total necrosis with the amount of atrophy:

Total Tissue Loss = Necrosis + Atrophy

The total volume of damage per brain was calculated by multiplying the total tissue loss per section by the distance between the sections:

Volume of damage (mm^3) = $\sum$sectional tissue loss × distance between sections (1 mm)

To control for any inter-animal brain size variability, and to control for any differences in brain sizes due to variable tissue processing, all values were expressed as a percentage of the contralateral hemisphere:

Normalized Value = Volume of damage (mm^3)/Volume of Contralateral Hemisphere

Statistics

Differences between groups on the Bederson neurological scale were analyzed using the **Kruskal-Wallis** test for non-parametric data with more than 2 groups. Post-hoc differences between individual groups were analyzed with the **Wilcoxon-Ranksum** test. Results were deemed to be significant for all tests if $p<0.05$.

Water consumption and weight gain differences between groups were analyzed with an **ANOVA** for parametric data, and a post-hoc Scheffé's test.

Differences between groups in the level of blood oxygen attained, and differences between groups in the size of the contralateral hemisphere were analyzed by an **ANOVA**. As well, any differences between groups in the physiological parameters that were not intentionally varied were analyzed with **ANOVA.**

An analysis of the normalized infarct sizes as a proportion of the contralateral hemisphere was done with the **Kruskal-Wallis** test for non-parametric data, post-hoc **Wilcoxon-Ranksum**.

A section by section analysis was undertaken to reveal where in the brain any effects of hyperoxemia occurred. This analysis was done with the **Kruskall-Wallis** test for non-parametric data, post-hoc **Wilcoxon-Ranksum**.

To examine whether there is a relationship between the level of blood oxygen during ischemia and the size of infarct, both a **regression** analysis and a **Spearman's Ranksum correlation** test were done to plot the size of infarct against intra-ischemic PO_2 for the normoxemic and intra-ischemic hyperoxemia groups.

Because it is valuable to know whether the behavioral outcome matched the size of infarct, a correlation was done between the Bederson Neurological Score and the normalized volumes of infarct for each rat using **Spearman's Ranksum correlation** test.

CHAPTER THREE: RESULTS

Mortality

Two rats died before the 14 day survival period was complete. The first rat, subjected to normoxia during and after his stroke, died on the second night after developing very labored breathing. The second rat, subjected to reperfusion hyperoxemia, also developed severe breathing problems and died on the third night post-surgery.

One rat, subjected to intra-ischemic hyperoxemia was euthanized 2 days after surgery. This rat had also developed severely labored breathing. It is the opinion of this experimenter that the breathing complications in all 3 rats arose as a result of tracheal damage and subsequent tracheal or laryngeal edema incurred during intubation, and were not due to any neurological effects of the stroke. There were no significant differences in mortality between groups.

Physiological Parameters

There were no statistical differences between groups in any of the controlled physiological parameters during the ischemic and the reperfusion periods (table 3).

Blood Oxygen Levels

The average hyperoxemia level induced in each of the treatment conditions was highly significant ($p < 0.0001$) when compared to the normoxemic control group. There were no significant differences between any groups in the normoxemia conditions. The normoxemic control group experienced an average arterial PO_2 of 98.9 ± 4.0 mm Hg (mean ± std) during their stroke and PO_2 of 99.6 ± 6.1 mm Hg after their stroke. The low-intra-ischemic hyperoxemia group displayed an average arterial PO_2 of 196.1 ± 25.9

Parameter	Group				
	Normoxic	Ischemic ↑O_2	Ischemic ↑↑O_2	Reper. ↑O_2	I+R ↑O_2
Ischemic PCO_2 (mm Hg)	35.5 ± 2.1	35.7 ± 1.9	35.1 ± 1.4	35.6 ± 1.4	34.8 ± 0.9
Reperfusion PCO_2	37.4 ± 1.5	37.1 ± 1.2	37.7 ± 1.7	37.7 ± 1.1	36.5 ± 1.8
Ischemic pH	7.40 ± 0.01	7.41 ± 0.02	7.41 ± 0.01	7.40 ± 0.01	7.41 ± 0.01
Reperfusion pH	7.38 ± 0.01	7.39 ± 0.01	7.39 ± 0.02	7.38 ± 0.01	7.38 ± 0.02
Ischemic glucose (mM)	6.2 ± 0.7	5.9 ± 0.9	6.2 ± .6	6.0 ± 0.8	6.2 ± 0.7
Reperfusion glucose	5.2 ± 0.6	5.2 ± 0.6	5.6 ± 0.8	5.1 ± 0.5	5.3 ± 0.4
Ischemic hematocrit (%)	43 ± 2	42 ± 2	41 ± 1	43 ± 1	41 ± 1
Reperfusion hematocrit	44 ± 2	43 ± 2	43 ± 1	43 ± 2	42 ± 1
Ischemic brain temp (°C)	37.0 ± 0.04	37.0 ± 0.03	37.0 ± 0.04	37.0 ± 0.02	37.0 ± 0.04
Reperfusion brain temp	37.0 ± 0.08	37.0 ± 0.04	37.0 ± 0.03	37.0 ± 0.05	37.0 ± 0.03
Ischemic body temp	37.3 ± 0.3	37.3 ± 0.1	37.5 ± 0.4	37.4 ± 0.3	37.5 ± 0.4
Reperfusion body temp	37.3 ± 0.2	37.2 ± 0.1	37.4 ± 0.3	37.3 ± 0.3	37.4 ± 0.4
Ischemic BP (mm Hg)	60.6 ± 0.7	60.0 ± 1.3	61.0 ± 1.3	60.7 ± 0.9	61.0 ± 1.0
Reperfusion BP	76.5 ± 10.5	69.8 ± 5.6	74.7 ± 10.2	74.9 ± 10.3	74.2 ± 11.7

Table 3 - Average (mean ± std) physiological parameters during and after the stroke.

mm Hg during their stroke, and the high intra-ischemic hyperoxemia group exhibited an average arterial PO_2 of 312.2 ± 48.4 mm Hg during their stroke. The difference in PO_2 levels between the low and high intra-ischemic hyperoxemia groups was also highly significant ($p<0.0001$), allowing the analysis of 2 different levels of intra-ischemic hyperoxemia. The reperfusion hyperoxemia group experienced average arterial PO_2 after their stroke of 261.3 ± 32.3 mm Hg. The intra-ischemic and reperfusion hyperoxemia group displayed average arterial PO_2 levels of 249.4 ± 42.3 mm Hg and 260.6 ± 30.9 mm Hg during and after their stroke, respectively (table 4, figure 7).

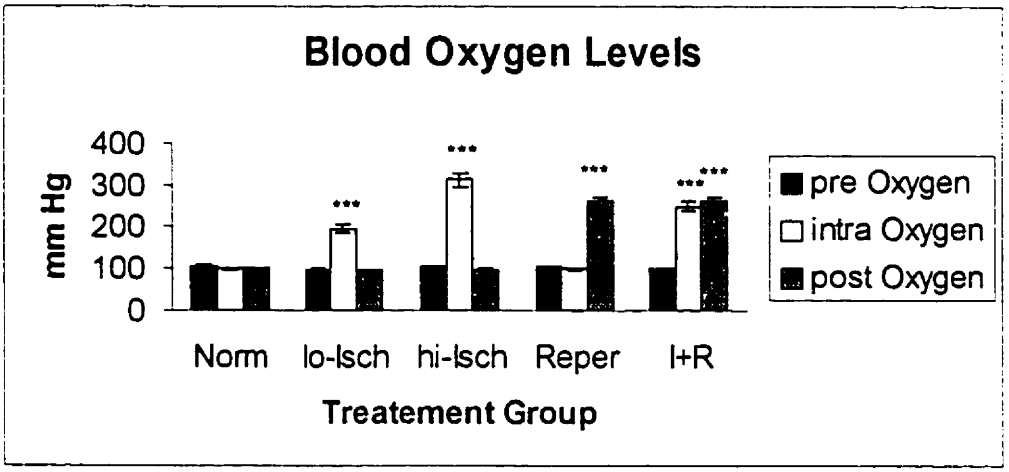

Figure 7 - Average blood oxygen levels before, during, and after the stroke. For this and all subsequent graphs, Norm = normoxemia; lo-Isch = low intra-ischemic hyperoxemia; hi-Isch = high intra-ischemic hyperoxemia; Reper = reperfusion hyperoxemia; I+R = intra-ischemic plus reperfusion hyperoxemia. Error bars represent standand error of the mean. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Group	Arterial PO_2 (mm Hg)		
	Pre-Ischemia	Intra-Ischemia	Post-Ischemia
Normoxemia	103.5 ± 13.2	98.9 ± 4.0	99.6 ± 6.1
Low Ischemic Hyperoxemia	94.0 ± 11.8	196.1 ± 25.9	94.4 ± 7.4
High Ischemic Hyperoxemia	104.6 ± 6.3	312.2 ± 48.4	96.4 ± 5.5
Reperfusion Hyperoxemia	103.9 ± 9.2	99.5 ± 3.7	261.3 ± 32.3
Ischemia plus Reperfusion Hyperoxemia	98.8 ± 6.9	249.4 ± 42.3	260.6 ± 30.9

Table 4 - Average (mean ± std) blood oxygen levels attained during and after the stroke.

Behavior

Bederson Neurological Scores

Those rats that experienced normal blood oxygen levels during and after their strokes had an average Bederson neurological score of 2.4. Those rats that experienced intra-ischemic hyperoxemia displayed significantly improved neurological scores when compared to this normoxemic group. Animals in the low intra-ischemic hyperoxemia

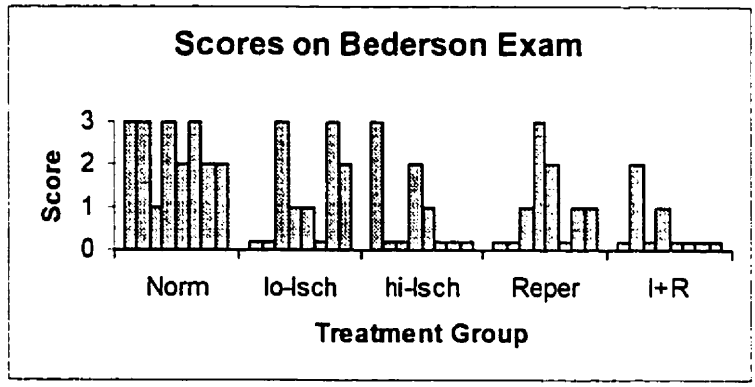

Figure 8 - Individual Bederson neurological scores for each group.

group had an average score of 1.2 ($p = 0.046$), and animals in the high intra-ischemic hyperoxemia group had an average score of 0.75 ($p = 0.012$). With these data, there is a trend towards improved neurological scores with higher levels of intra-ischemic hyperoxemia (figure 8, figure 9).

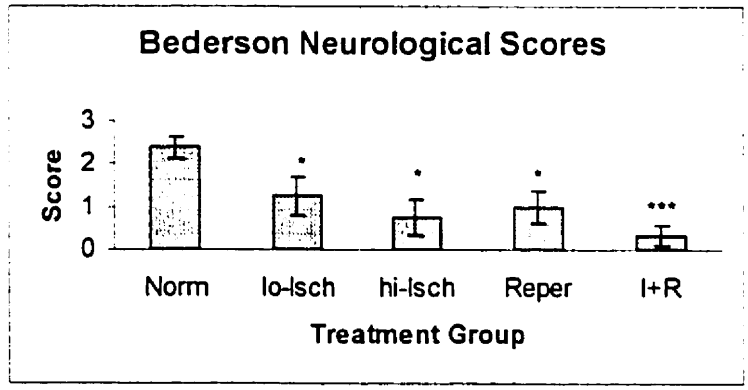

Figure 9 - Average Bederson neurological scores for each group.

Reperfusion hyperoxemia also resulted in significantly improved neurological scores when compared to the normoxemic control group. In this treatment group, rats scored an average of 1 on the Bederson neurological scale ($p = 0.017$). These results challenge the theory of reperfusion injury due to the augmentation of oxygen derived free radicals, since there is increased substrate for the injury, but there is in fact less neurological damage.

From a medical point of view, however, the champion treatment was to induce hyperoxemia at the beginning of the stroke, and maintain that hyperoxemia throughout the reperfusion period. Animals who experienced intra-ischemic hyperoxemia and reperfusion hyperoxemia had an average Bederson neurological score of only 0.33 (p = 0.0008).

Post hoc analysis of any other differences between groups showed no significant differences on the Bederson neurological scale.

Water Consumption

Normoxemic rats drank an average of 158 ml of water during the first 7 days of their 14 day survival period (figure 10). Animals treated with low levels of intra-ischemic hyperoxemia and high levels of intra-ischemic hyperoxemia drank an average of 268 ml (p = 0.069) and 174 ml (p = 0.995) respectively. Although not statistically significant, these data show a trend towards more water consumption during the survival period with intra-ischemic hyperoxemia.

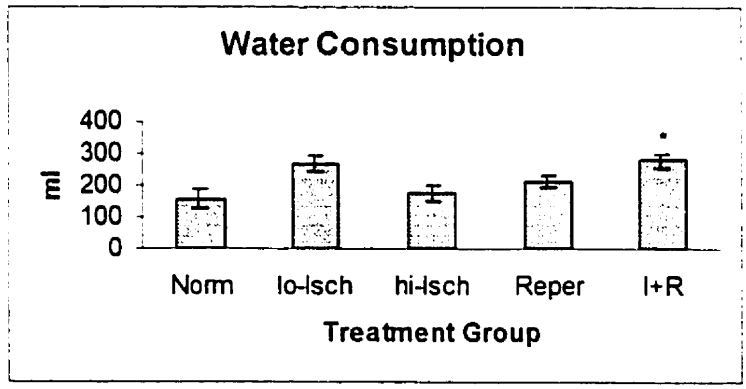

Figure 10 - Average water consumption for each group.

Reperfusion hyperoxemia treated rats, on average, also drank more water than the normoxemic control group, although the results were, similarly, not significant.

Reperfusion hyperoxemia rats drank an average of 213 ml ($p = 0.674$) of water during the first 7 days after their surgery.

Intra-ischemic plus reperfusion hyperoxemia treatment resulted in a significant increase in average water consumption. These rats drank an average of 280 ml ($p = 0.028$) of water in the first 7 days after their surgery (figure 10).

Post hoc analysis of any other differences between groups revealed no statistical significance.

Weight gain/loss

Normoxemic control rats lost an average of 22 grams during their 14 day survival period (figure 11). It was observed that these rats either did not attempt to eat, or had trouble swallowing.

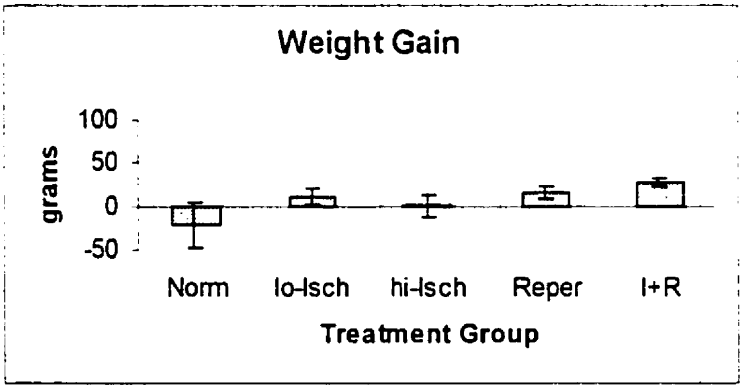

Figure 11 - Average weight gain/loss for each group.

The low intra-ischemic hyperoxemia group gained an average of 11 grams ($p = 0.615$) during their survival, and the high intra-ischemic hyperoxemia group gained an average of 1 gram during their survival ($p = 0.847$). The reperfusion hyperoxemia group gained an average of 16 grams during their survival ($p = 0.467$). Those rats who were exposed to hyperoxemia both during and after their strokes gained an average of 26 grams ($p = 0.208$).

There were no significant differences between any of the treatment groups compared to the control group when considering the change in weight from the day of surgery to the end of their survival periods. Although there was a trend toward greater weight gain with the hyperoxemia treated animals, there is no significance probably due to the high degree of variability displayed by the animals.

Post hoc analysis for any other statistical differences between treatment groups revealed no significance.

Qualitative Description of Brain Damage

Damage occurred exclusively in the lateral portions of the brain. Medial structures, such as the septal nuclei, most of the thalamus, and most of the hypothalamus, were saved from necrosis with this stroke model. As well, the hippocampus was uniformly saved, even in the rat with the most extensive damage.

Damage occurred in the cortex. Cortical damage occurred in the insular cortex (both granular and agranular), the frontal cortex, the parietal cortex, the perirhinal cortex, and sometimes in the temporal cortex. Most cortical damage corresponded to the area around the rhinal fissure, where the middle cerebral artery lies. Subcortical damage occurred in structures such as the olfactory nucleus, the nucleus accumbens, the medial forebrain bundle, the caudate/putamen, the claustrum, the globus pallidus, the pallidum, the Nucleus of Meynert, and the amygdaloid. The lateral nucleus of the hypothalamus was sometimes damaged. Thalamic nuclei such as the VL, VM, VPL, and VPM, and the lateral reticular nucleus of the thalamus were also sometimes damaged. The internal capsule was injured in some animals.

Histology – Volume Analysis

Absolute Volume of Necrosis vs. Proportional Necrosis

In most cases examined, it was found that expressing the neuronal damage as a percentage of the contralateral hemisphere instead of the absolute volume of necrosis in mm^3 resulted in lower probabilities that the differences were due to chance alone (p). For example, when considering the volume of subcortical necrosis in mm^3, an ANOVA between groups resulted in a p value of 0.0212; a Kruskall Wallis test of the same data when expressed as a percentage of the contralateral hemisphere resulted in a p value of 0.0140. As well, when considering the amount of atrophy in the affected hemisphere, an ANOVA between groups resulted in a p value of 0.0676 (not significant), whereas a Kruskall-Wallis test of the same data expressed as a percentage of the contralateral hemisphere resulted in a p value of 0.0591 (approaching significance). This finding concurs with previous results in the literature that show a superiority of percentage over absolute volume of damage as a measurement[61]. Because of this finding, and the desire to control for variable brain sizes in animals, all histological results, except for the contralateral hemisphere size, have been statistically analyzed as a percentage of the contralateral hemisphere, using the Kruskall –Wallis test for non-parametric data.

Contralateral Hemisphere Size

As all brain damage is expressed as a percentage of the contralateral hemisphere, it is important to verify that there are no significant differences between treatment groups in this parameter (figure 12). Any significant differences between groups in contralateral hemisphere size, whether caused by the stroke, the treatment, random chance, or the age of animals in the group, would influence the normalized values of damage when

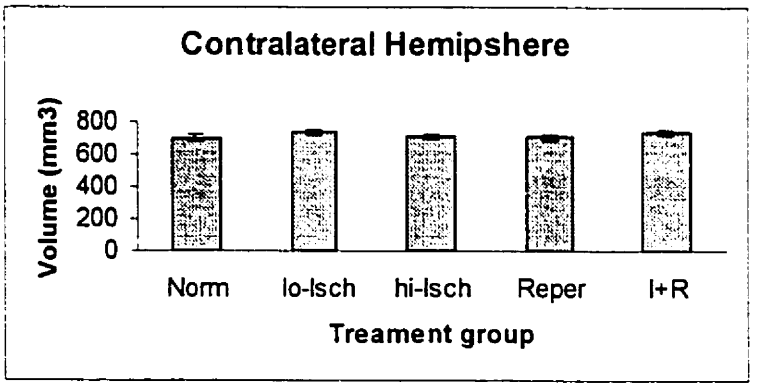

Figure 12 - Average volume of the contralateral hemisphere for each group.

expressed as a proportion, confounding the results. An ANOVA on the size of the contralateral hemisphere revealed no significant differences between groups ($p = 0.459$).

Infarcts

An example of a stained coronal section is shown in figure 13. Note the cortical and the subcortical infarction. As well, note that the hemisphere with the damage is smaller in area than the unaffected hemisphere. The border of the infarct is quite

Figure 13 - Example of a stained coronal section.

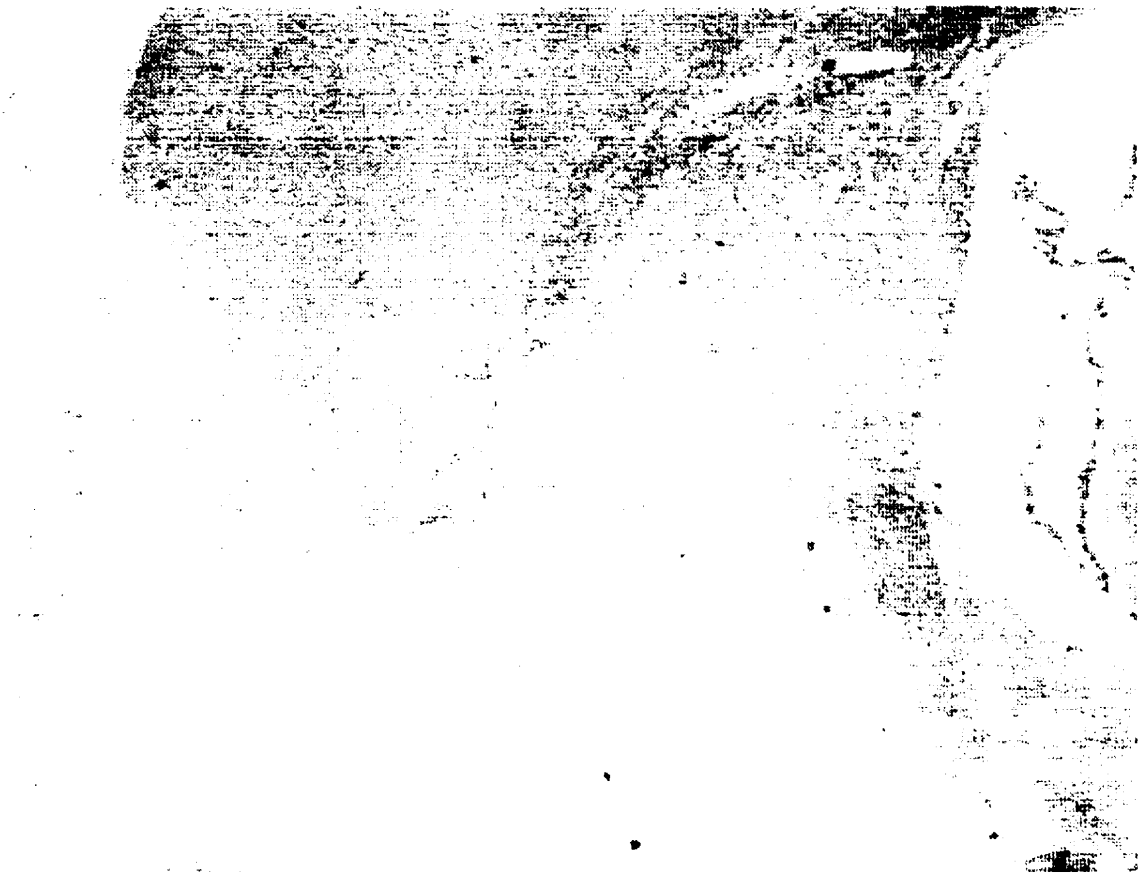

Figure 14 - Higher resolution of stained section, showing border of infarct.

demarcated, as can be seen in the higher resolution of figure 14. This allows the quantification of infarct size to be rather exact.

Cortical Damage

Rats who were exposed to normoxemia during and after their stroke displayed an average of 45 mm^3 of cortical damage, or 6.5% of the total volume of the contralateral hemisphere (figure 15). Those rats who experienced intra-ischemic hyperoxemia in the low range exhibited less cortical damage, measuring an average of 17 mm^3, or 2.3% (p = 0.0223) of the volume of the contralateral hemisphere. Animals who were treated with high levels of intra-ischemic hyperoxemia also exhibited less cortical damage, exhibiting an average of 16 mm^3, or 2.1% (p = 0.0484) of the volume of the contralateral

hemisphere. Both of these treatment groups enjoyed significantly less cortical damage when compared to the normoxemic control group.

Reperfusion hyperoxemia also resulted in less cortical damage, measuring an average of 18 mm^3, or 2.7% ($p = 0.0820$) of the volume of the contralateral hemisphere. This is another challenge to the notion of reperfusion injury due to oxygen-derived free radicals – although more oxygen is present in the blood that returns to ischemic tissue, cortical damage is less than the normoxemic, control group.

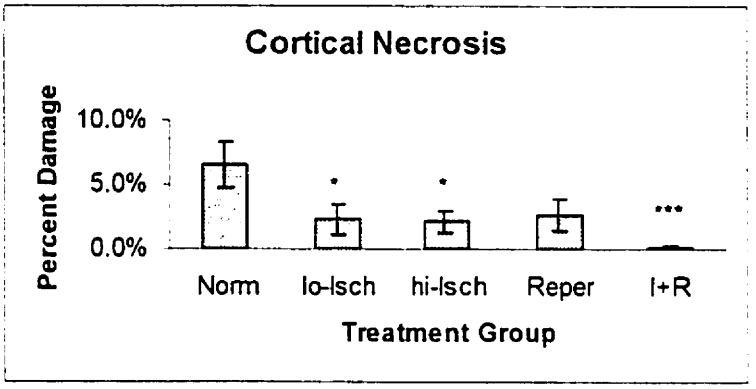

Figure 15 - Average volume of cortical damage for each group.

Intra-ischemic hyperoxemia combined with reperfusion hyperoxemia resulted in dramatic, consistently improved neurological outcome, with cortical damage reduced to an average of only 1 mm^3, or only 0.2% ($p = 0.0005$) of the volume of the contralateral hemisphere for this treatment group.

A *post hoc* analysis for any other significant differences in cortical damage between treatment groups revealed a couple of interesting things. The difference in the volume of cortical damage between the high intra-ischemic hyperoxemia treatment group and the intra-ischemic plus reperfusion hyperoxemia group was statistically significant ($p = 0.0418$). As well, the difference in the volume of cortical damage between the reperfusion hyperoxemia treatment group and the intra-ischemic plus reperfusion

hyperoxemia treatment group was statistically significant ($p = 0.0389$). These results indicate the drastic improvement seen with the intra-ischemic plus reperfusion hyperoxemia treatment is statistically superior even to other hyperoxemia treatments, which also show improvement over the normoxic control group.

Subcortical Damage

Control rats with intra-ischemic and reperfusion normoxemia were afflicted with an average of 60 mm^3 of subcortical necrosis, representing 8% of the total volume of the contralateral hemisphere (figure 16). Rats treated with intra-ischemic hyperoxemia into the low and high range exhibited subcortical necrosis of 45 mm^3, or 6% ($p = 0.1736$), and 47 mm^3, or 7% ($p = 0.1392$) respectively. Although these differences between treatments

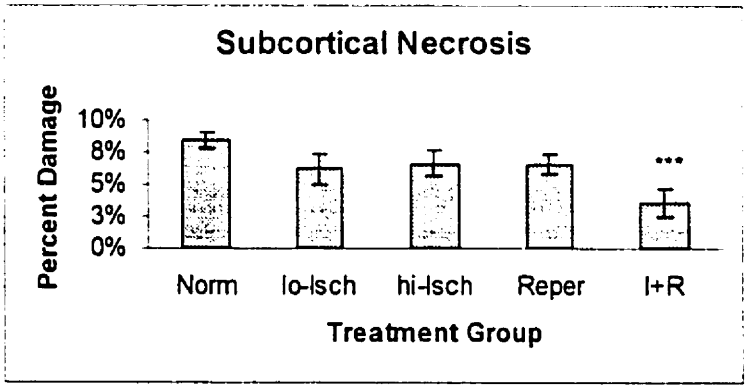

Figure 16 - Average volume of subcortical damage for each group.

were not significant, there is a trend towards less subcortical damage in the intra-ischemic hyperoxemia treatment groups when compared to the normoxemic group.

While the reperfusion hyperoxemia group did not display a significant reduction in the amount of subcortical necrosis ($p = 0.0821$), there was a trend towards less damage, with only 47 mm^3 of the affected hemisphere infarcted, or 7% of the volume of the contralateral hemisphere. This represents another challenge to the theory of reperfusion injury due to oxygen-derived free radicals, as subcortical damage is not

increased, but rather reduced.

Once again those rats who were exposed to both intra-ischemic and reperfusion hyperoxemia fared the best, displaying significantly less subcortical damage when compared to the normoxemic group. Rats in this treatment group exhibited subcortical necrosis of only 27 mm^3, or 4% ($p = 0.0007$) of the total volume of the contralateral hemisphere.

A *post hoc* analysis of any other differences in subcortical damage between treatment groups revealed that the drastic improvement in the intra-ischemic plus reperfusion hyperoxemia treatment group was significantly different from the reperfusion hyperoxemia only treatment group ($p = 0.0201$). This shows that the addition of beneficial intra-ischemic hyperoxemia to the reperfusion hyperoxemia treatment creates a condition that results in markedly reduced subcortical damage when compared to the control group.

Total Necrosis

The total amount of tissue lost to necrosis is the cortical damage plus the subcortical damage. Those animals that experienced normal blood oxygen levels during and after their stroke suffered an average of 105 mm^3 of total necrosis in the affected hemisphere, or 15% of the volume of the contralateral hemisphere (figure 17). The intra-ischemic hyperoxemia treatment groups exhibited significantly less total necrosis when compared to the control group. Those rats who experienced low intra-ischemic hyperoxemia lost an average of 62 mm^3, or 8% ($p = 0.0494$) of the volume of the contralateral hemisphere to necrosis. The high intra-ischemic hyperoxemia treatment resulted in an average necrosis of 63 mm^3, or 9% ($p = 0.0346$) of the volume of the

contralateral hemisphere.

Reperfusion hyperoxemia produced an average necrosis of 65 mm^3, or 9 % (p = 0.0696) of the volume of the contralateral hemisphere. The theory of reperfusion injury would predict that this group would exhibit significantly *greater* necrosis than a normoxic control group, due to a greater substrate for the production of oxygen derived free radicals. The fact that reperfusion hyperoxemia produces *less* damage directly calls

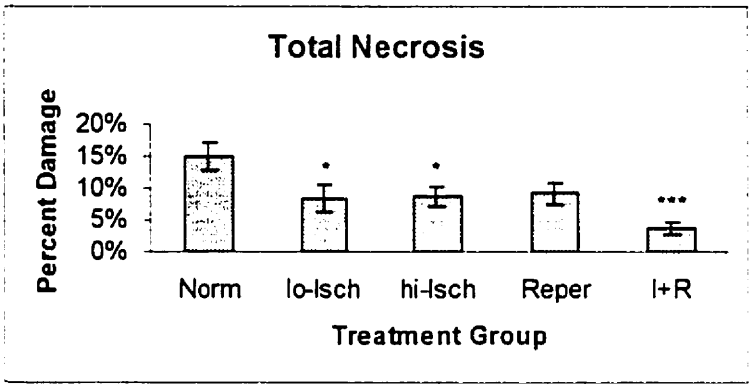

Figure 17 - Average volume of total necrosis for each group.

into question this theory of reperfusion injury.

Consistent with the previous data, those rats that experienced intra-ischemic and reperfusion hyperoxemia enjoyed a total necrosis of only 28 mm^3 , or 4% (p = 0.0003) of the volume of the contralateral hemisphere.

A *post hoc* analysis for any other significant differences in total necrosis between treatment groups revealed that the intra-ischemic plus reperfusion hyperoxemia group had significantly less damage than the high intra-ischemic hyperoxemia only group (p = 0.0278). This suggests that although the effects of intra-ischemic hyperoxemia significantly mitigate neuronal damage, the addition of reperfusion hyperoxemia to this treatment makes the outcome significantly more beneficial. As well, it was found that the

improvement found with the intra-ischemic plus reperfusion hyperoxemia treatment was significantly different from the reperfusion hyperoxemia only treatment ($p = 0.0075$). This indicates that the addition of intra-ischemic hyperoxemia to reperfusion hyperoxemia is significantly more beneficial than reperfusion hyperoxemia alone.

Atrophy

Tissue is lost to the removal of dead tissue by macrophages, and also to shrinkage due to trans-synaptic atrophy. The former is present only in the infarcted hemisphere. The latter, however, would be expected to influence both hemispheres to some degree, because of commissural fibers of the brain. The normoxic control group lost an average of 70 mm^3 from the affected hemisphere, or 10% of the volume of the contralateral hemisphere (figure 18). A Kruskall-Wallis analysis revealed that none of the hyperoxemia treatment groups displayed significantly less atrophy when compared to the normoxemic control group ($p = 0.0591$). Low levels of intra-ischemic hyperoxemia produced an average of 43 mm^3 lost from the ipsilateral hemisphere, equivalent to 6% of

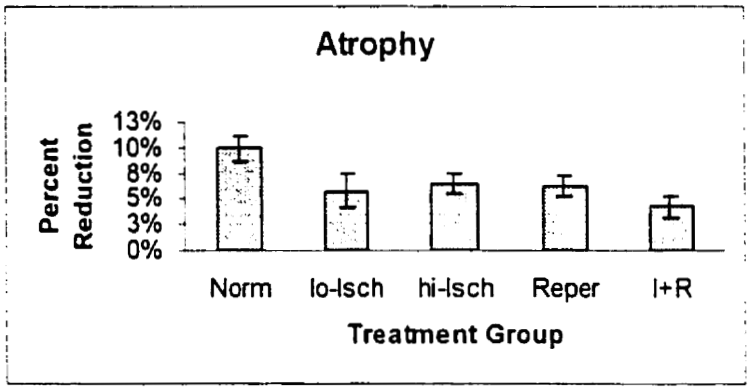

Figure 18 - Average volume of atrophy for each group.

the volume of the contralateral hemisphere. High intra-ischemic hyperoxemia resulted in an average atrophy of 47 mm^3 of the ipsilateral hemisphere, or 6% of the volume of the contralateral hemisphere.

The reperfusion hyperoxemia treatment displayed an average of 44 mm^3 lost from the stroked hemisphere, or 6% of the volume of the contralateral hemisphere.

While not significant for this dependent variable, the intra-ischemic plus reperfusion hyperoxemia group consistently showed the best outcome. Animals in this group lost an average of only 32 mm^3 of tissue from the ipsilateral hemisphere, or 4% of the volume of the contralateral hemisphere.

Total Tissue Lost

The total amount of tissue lost, as mentioned above, is the total amount of necrosis plus the tissue lost to atrophy. The normoxic control group suffered an average of 175 mm^3 of total tissue damage, or 25% of the volume of the contralateral hemisphere (figure 19). The intra-ischemic hyperoxemia treatments showed significantly less total damage when compared to this control group. Rats exposed to low levels of intra-ischemic hyperoxemia had an average tissue loss of 104 mm^3, or 14% ($p = 0.0413$) of the volume of the contralateral hemisphere. High intra-ischemic hyperoxemia resulted in an

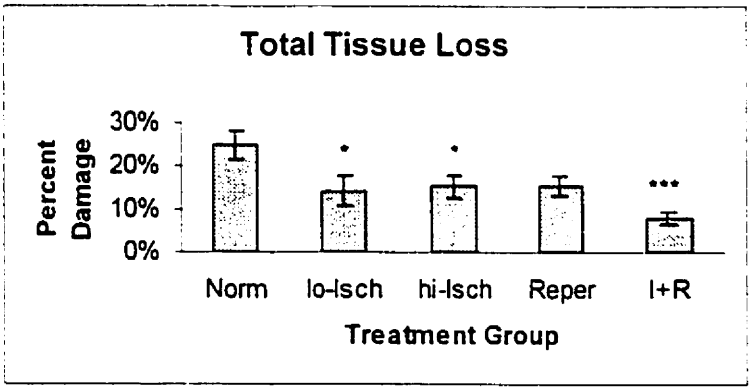

Figure 19 - Average volume of total tissue loss for each group.

average total tissue loss of 110 mm^3, or 15% ($p = 0.0346$) of the contralateral hemisphere.

The reperfusion hyperoxemia group also displayed less total damage when compared to the control group, losing an average of 110 mm^3 from the affected

hemisphere, or 16% ($p = 0.0588$) of the volume of the contralateral hemisphere. Since this difference is approaching significance, this challenges the theory of oxygen-derived free radical reperfusion injury, which would have predicted a significant *increase* in the amount of total damage.

A significant reduction in the total amount of tissue lost was evident on the intra-ischemic plus reperfusion hyperoxemia group. These animals lost an average of only 59 mm^3 from the ischemic hemisphere, or 8% ($p = 0.0009$) of the volume of the contralateral hemisphere.

An analysis done *post hoc* for any other differences between groups revealed that the beneficial effects seen with high intra-ischemic hyperoxemia significantly improves if reperfusion hyperoxemia is added to the treatment ($p = 0.0115$). Similarly, it was revealed that the beneficial effects of reperfusion hyperoxemia can be significantly improved with the addition of intra-ischemic hyperoxemia to the treatment ($p = 0.0167$).

Histology – Sectional Analysis

Cortex

The analysis of where, along the longitudinal antero-posterior axis of the rat brain, the beneficial effects of hyperoxemia occurred will begin with the cortex. In the normoxemic rat, the most cortical damage was displayed in the middle sections of the brain, specifically from section 10.2 (anterior) to 6.2 (posterior), with an average necrosis of approximately 11% of the corresponding contralateral hemispheric area. In the low intra-ischemic hyperoxemia group, the most cortical damage was represented in sections 10.2 to 7.2, averaging necrosis of about 5% of the corresponding contralateral hemisphere section. There was significantly less cortical damage in the low intra-

ischemic group when compared to the control group at sections **11.2** ($p = 0.0393$), **10.2** ($p = 0.0393$), **8.2** ($p = 0.0385$), and **6.2** ($p = 0.0073$) (figure 20).

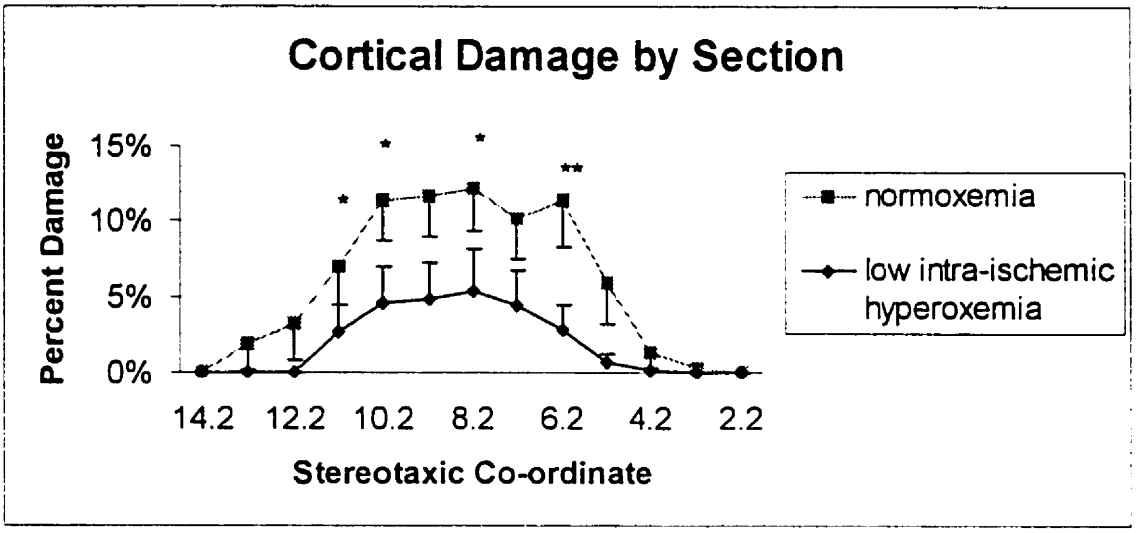

Figure 20 - Sectional analysis of cortical necrosis for the normoxemic and low intra-ischemic hyperoxemia groups.

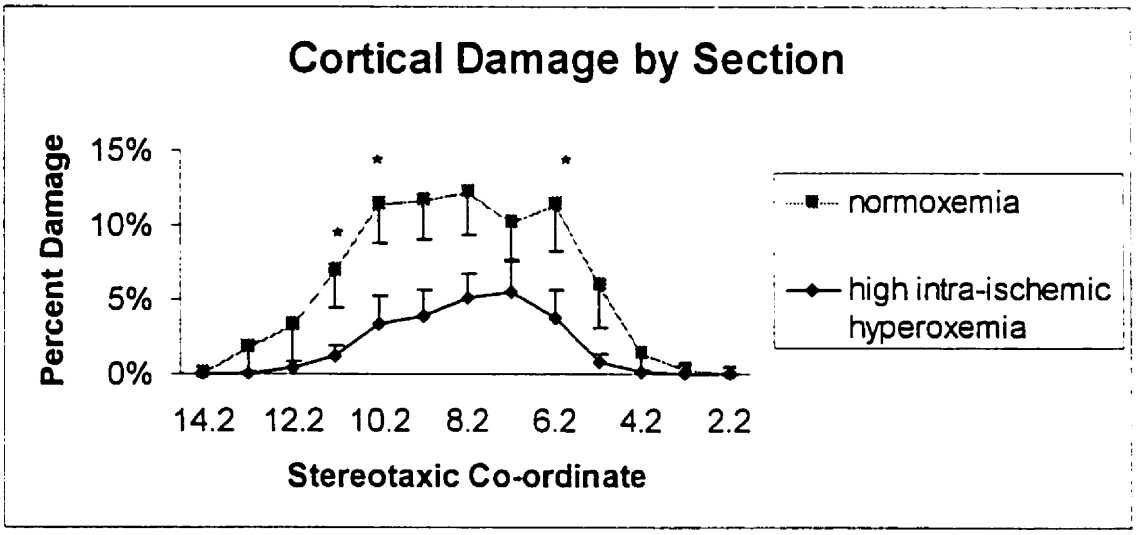

Figure 21 - Sectional analysis of cortical necrosis for the normoxemic and high intra-ischemic hyperoxemia groups.

The high intra-ischemic hyperoxemia group (figure 21) displayed the most cortical damage in the middle sections 8.2 (anterior) to 6.2 (posterior). In these sections the average cortical necrosis was about 5% of the corresponding contralateral hemisphere

section. Significant differences between this treatment group and the normoxemic control group occurred at sections **11.2** ($p = 0.0171$), **10.2** ($p = 0.0278$) and **6.2** ($p = 0.0193$). Together, these analyses indicate that hyperoxemia during ischemia protects the cortex over the entire hemisphere.

Those rats who experienced reperfusion hyperoxemia (figure 22) displayed the most cortical damage between sections 10.2 (anterior) to 6.2 (posterior). At these levels, damage was between 5 – 6% of the corresponding contralateral section. Like the high intra-ischemic hyperoxemia group, significant differences occurred between the reperfusion hyperoxemia group and the normoxemic control group at sections **11.2** ($p = 0.0174$), **10.2** ($p = 0.0423$), and **6.2** ($p = 0.0485$).

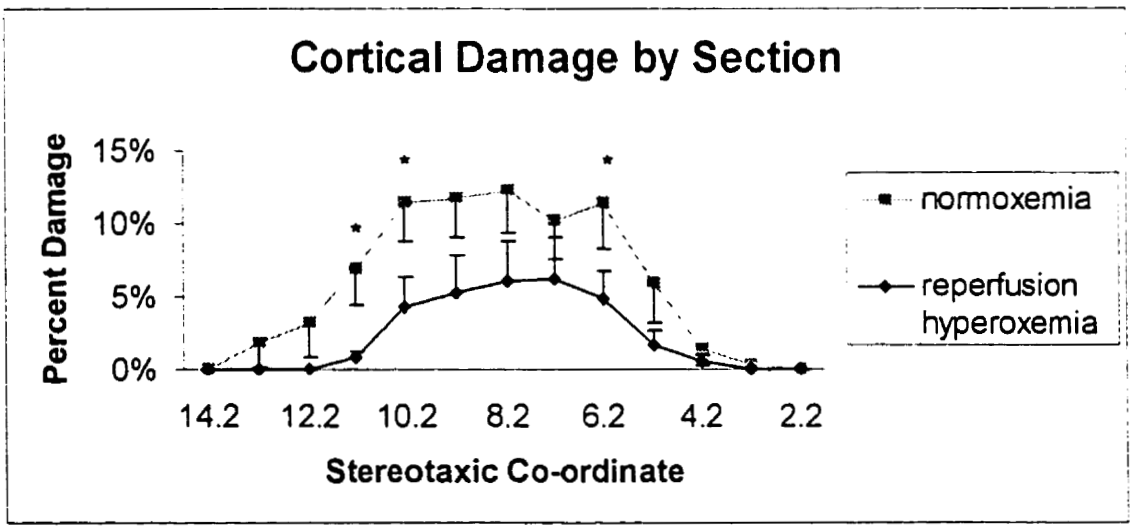

Figure 22 - Sectional analysis of cortical necrosis for the normoxemic and reperfusion hyperoxemia groups.

The intra-ischemic plus reperfusion hyperoxemia treatment resulted in negligible cortical damage anywhere in the brain (figure 23). The section with the most damage, although very slight, was 7.2, where there was an average necrosis of less than 1% of the corresponding contralateral hemisphere section. Highly significant differences occurred

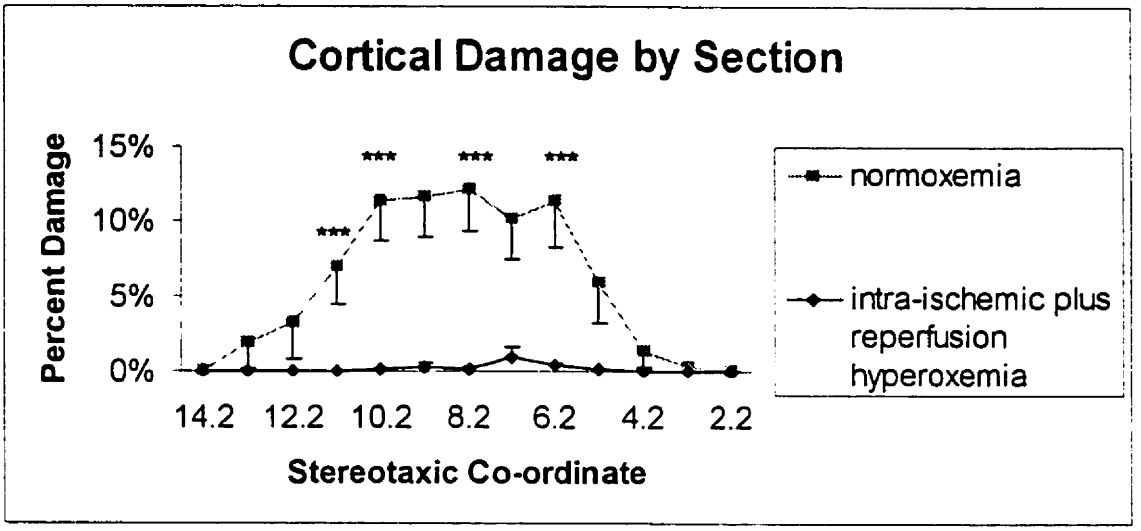

Figure 23 - Sectional analysis of cortical necrosis for the normoxemic and intra-ischemic plus reperfusion hyperoxemia groups.

between this treatment and the normoxemic group at levels **11.2** (p = 0.0004), **10.2** (p = 0.0008), **8.2** (p = 0.0005), and **6.2** (p = 0.0001).

It is important to verify here that although there are seemingly large differences in cortical damage between normoxemia and all the groups at levels 9.2 and 7.2, especially in the intra-ischemic plus reperfusion hyperoxemia group, the Kruskal-Wallis test for non-parametric data revealed that there were no significant differences between any groups at level 9.2 (p = 0.0640) or at level 7.2 (p = 0.1471). This non-significance precluded any statistically legal *post hoc* analyses for differences between individual groups.

Subcortex

Control rats displayed the most subcortical damage in sections 10.2 (anterior) to 6.2 (posterior). Average subcortical damage in these sections for the normoxemic group was about 22-25% of the corresponding contralateral hemisphere section. Low intra-ischemic hyperoxemia (figure 24) resulted in the most damage in sections 10.2 – 8.2,

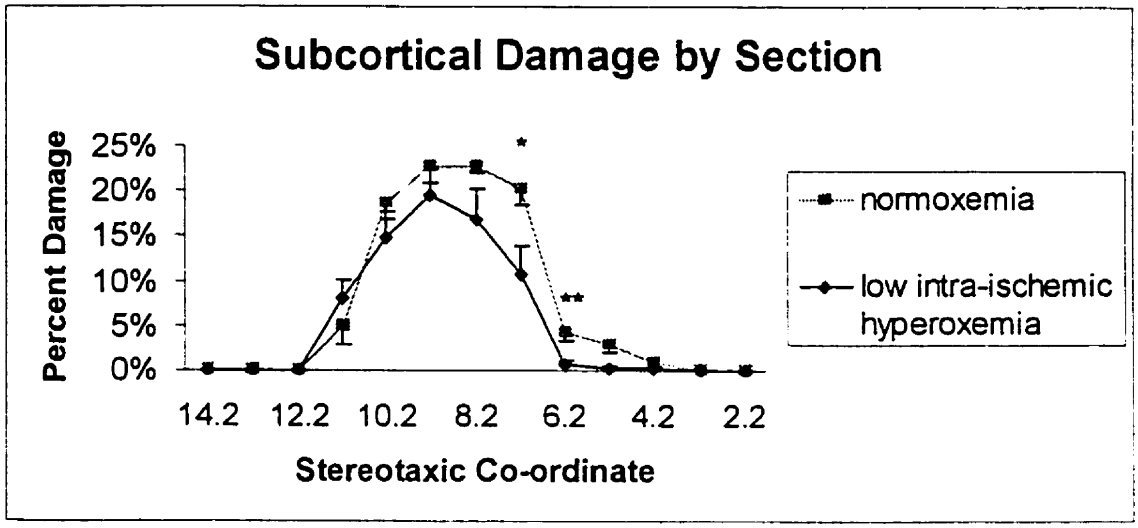

Figure 24 - Sectional analysis of subcortical necrosis for the normoxemic and low intra-ischemic hyperoxemia groups.

averaging about 18 % of the corresponding contralateral hemisphere section. The bulk of subcortical damage tapered off toward the posterior part of the brain, resulting in significant differences between the groups at sections **7.2** ($p = 0.0191$) and **6.2** ($p = 0.0028$).

Animals that experienced high levels of intra-ischemic hyperoxemia displayed reduced overall subcortical damage (figure 25), but the subcortical damage at all sections was not significantly different from the control group.

Similarly, the subcortical damage exhibited with the reperfusion hyperoxemia group (figure 26) was not significantly different at any section when compared to the normoxic control group.

Animals in the intra-ischemic plus reperfusion hyperoxemia group showed the most improvement (figure 27) when compared to the normoxemic group. Rats in this group exhibited the most subcortical damage in sections 10.2 – 7.2, averaging about 8% of the corresponding contralateral hemisphere. There were significant improvements in

subcortical damage at sections **10.2** ($p = 0.0015$), **8.2** ($p = 0.0015$), **7.2** ($p = 0.0009$), and **6.2** ($p = 0.0030$).

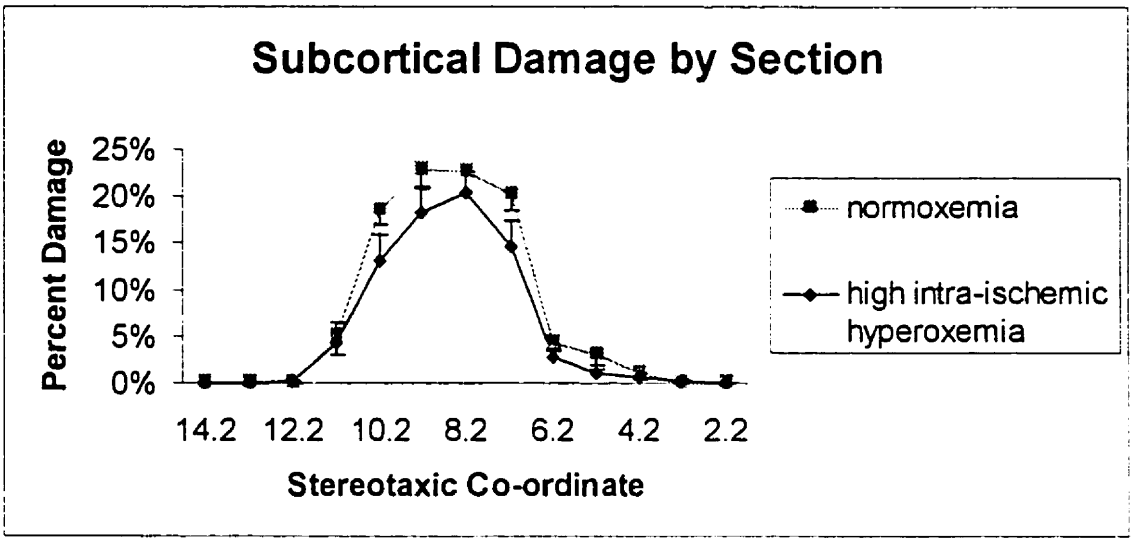

Figure 25 - Sectional analysis of subcortical damage for the normoxemic and high intra-ischemic hyperoxemia groups.

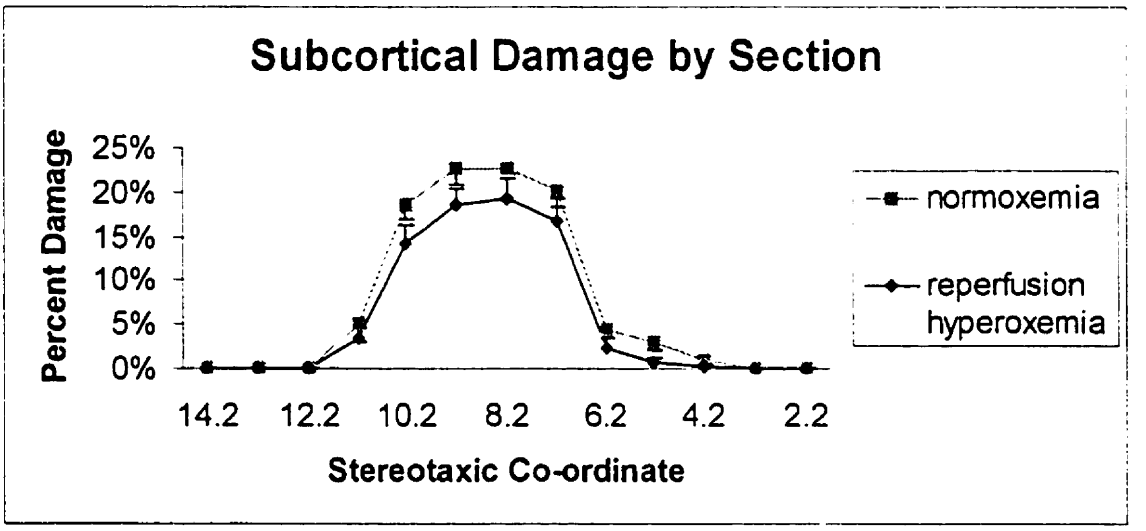

Figure 26 - Sectional analysis of subcortical damage for the normoxemic and reperfusion hyperoxemia groups.

Atrophy

The amount of brain atrophy was the greatest in the normoxemic control group. Like all other groups, the greatest amount of atrophy took place in the anterior sections of

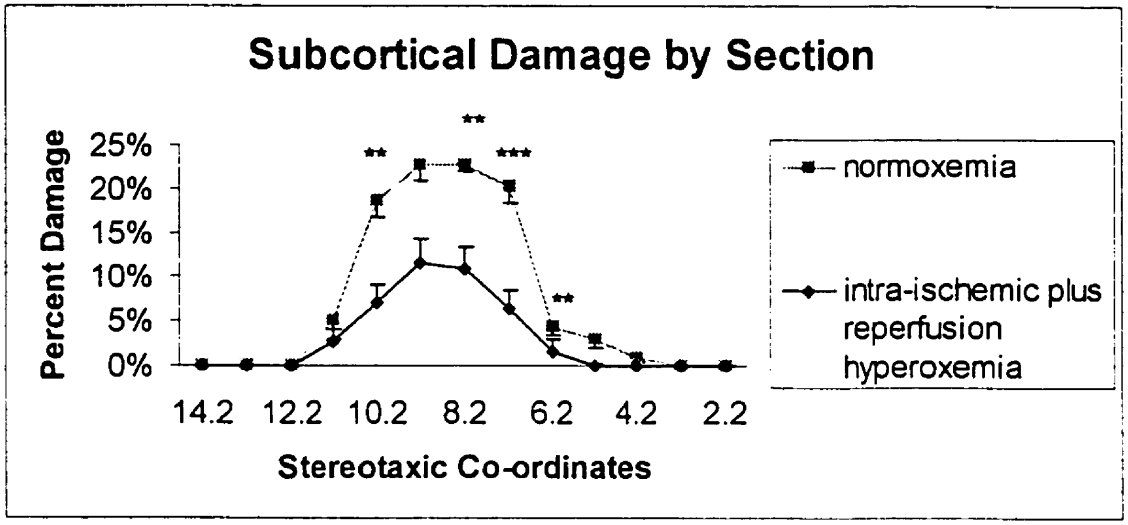

Figure 27 - Sectional analysis of subcortical necrosis for the normoxemic and intra-ischemic plus reperfusion hyperoxemia groups.

the brain, namely 14.2 to 11.2, and the middle sections of the brain, namely 10.2 to 7.2, where the average amount of atrophy was around 15% of the corresponding contralateral hemisphere section. The low intra-ischemic hyperoxemia group showed less overall atrophy (figure 28) in a uniform distribution along the antero-posterior brain axis, an

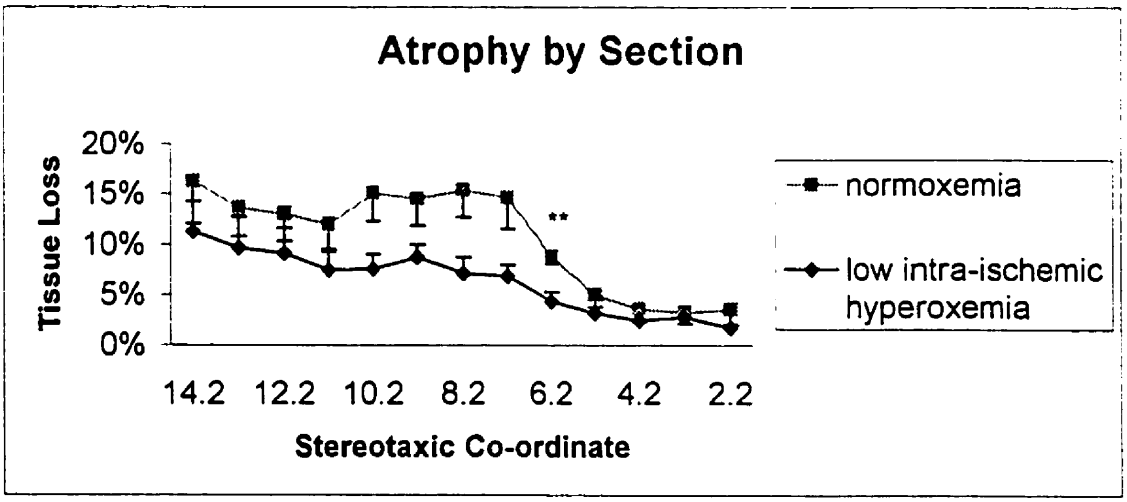

Figure 28 - Sectional analysis of atrophy for the normoxemic and low intra-ischemic hyperoxemia groups.

indication of hemispheric neuroprotection even at coronal levels in front of, and behind the infarct. In this group, the greatest tissue loss happened anteriorly and tapered off

toward the posterior end of the brain. A significant difference in the amount of atrophy between this group and the control group occurred at level **6.2** ($p = 0.0082$).

In the high intra-ischemic hyperoxemia group (figure 29), again most atrophy occurred anteriorly and tapered off toward the posterior end of the brain. The most atrophy in this group occurred at levels 14.2 to 12.2, where tissue loss averaged about 13% of the contralateral hemisphere. Significant reductions in atrophy when compared to the control group took place in levels **8.2** ($p = 0.0242$) and **6.2** ($p = 0.0039$).

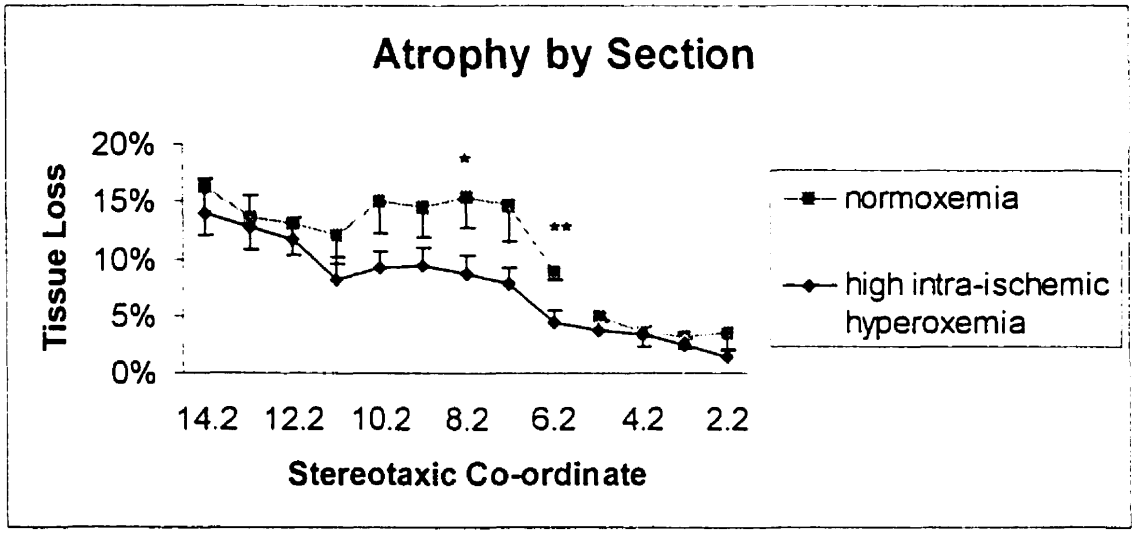

Figure 29 - Sectional analysis of atrophy for the normoxemic and high intra-ischemic hyperoxemia groups.

In the reperfusion hyperoxemia treatment (figure 30), there was overall less atrophy when compared to the normoxemic control group. Most of the atrophy again occurred anteriorly, tapering off toward the posterior end of the brain. A significant difference between this group and the control group took place at level **8.2** ($p = 0.0494$).

The intra-ischemic plus reperfusion hyperoxemia group (figure 31) displayed approximately the same amount of amount of atrophy as the other hyperoxemia treatment groups. As seen with the other treatments, most atrophy occurred anteriorly, but

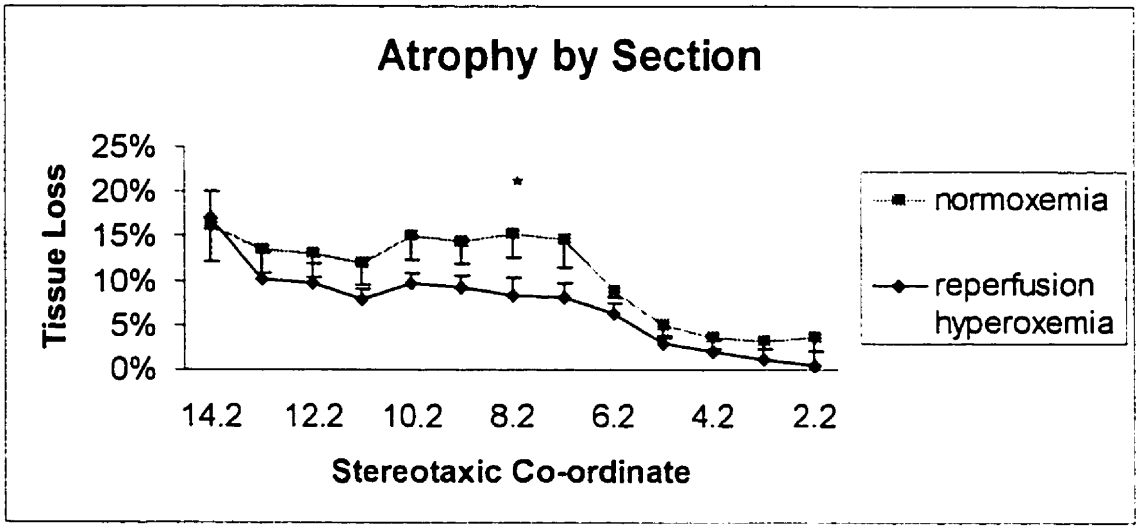

Figure 30 - Sectional analysis of atrophy for the normoxemic and reperfusion hyperoxemia groups.

remarkably in this group, neuroprotection was seen posteriorly, even in the occipital lobe, very remote from the infarct. Significant differences between this group and the normoxemic group occurred at levels **8.2** (p = 0.0049), **6.2** (p = 0.0060), and **2.2** (p = 0.0137).

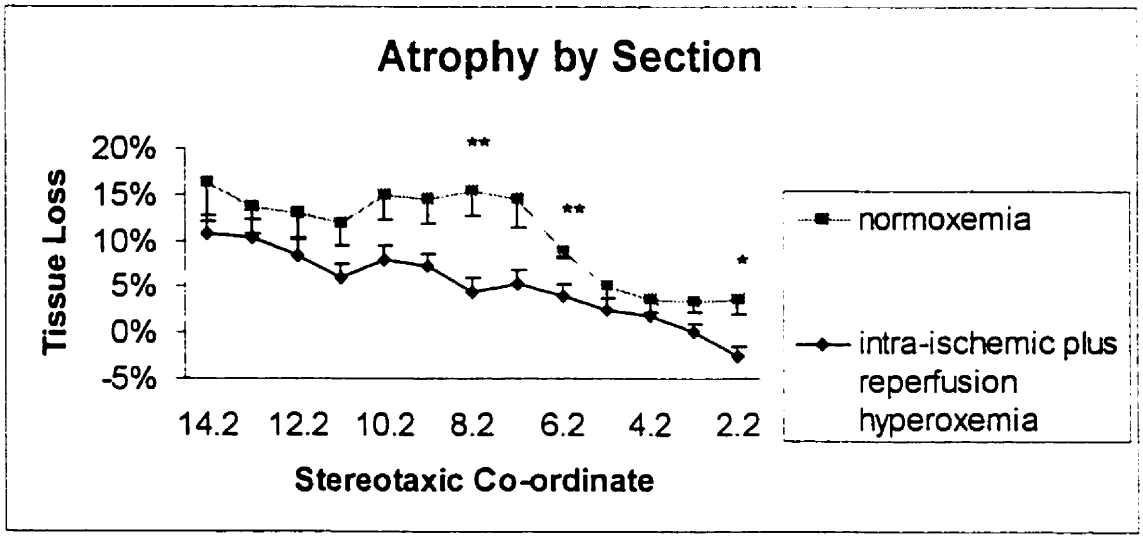

Figure 31 - Sectional analysis of atrophy for normoxemic and intra-ischemic plus reperfusion hyperoxemia groups.

Total Tissue Loss

As mentioned before, the total tissue lost is effectively a summary measure of brain damage, consisting of the sum of the necrotic plus tissue lost to atrophy. In the normoxemic control group (figure 32), rats lost the most tissue in the middle sections of the brain, namely sections 10.2 to 7.2, where an average of 50% of the corresponding contralateral hemisphere section was lost, a considerable amount. The low intra-ischemic hyperoxemia group lost less tissue overall than the control group, with the greatest tissue damage also occurring in middle sections 10.2 to 7.2, representing around 30% of the contralateral hemisphere. Significant differences between this treatment and the control group took place at levels **8.2** ($p = 0.0003$), **7.2** ($p = 0.0007$), and **6.2** ($p = 0.0007$).

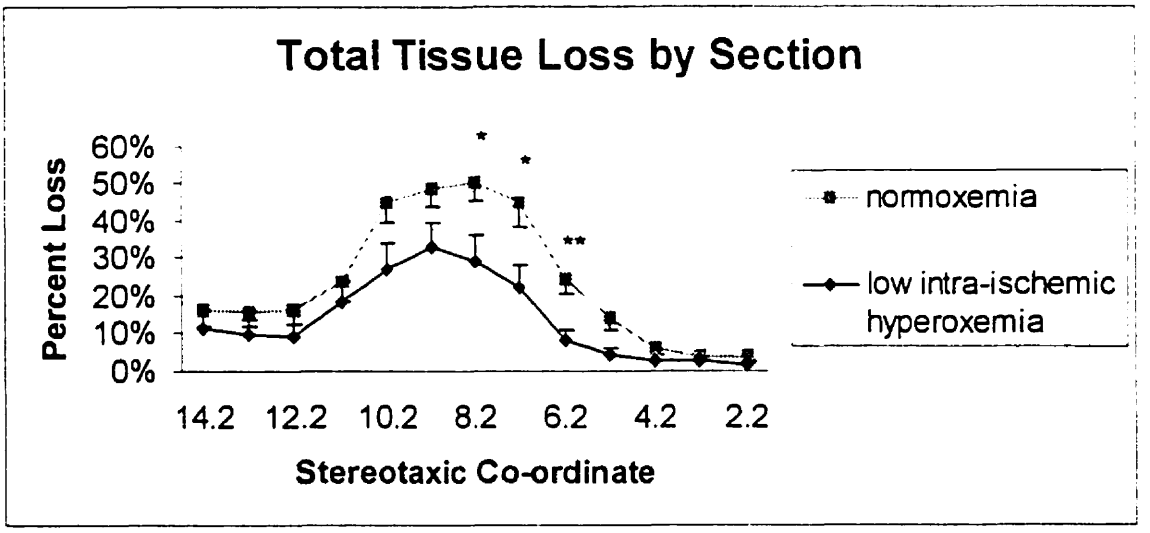

Figure 32 - Sectional analysis of total tissue loss for normoxemic and low intra-ischemic hyperoxemia groups.

In the high intra-ischemic hyperoxemia group (figure 33), there was again less overall damage when compared to the normoxemic control group. The greatest damage in this condition occurred in the middle sections 10.2 to 7.2, averaging around 30% of the contralateral hemisphere section. Significant differences between this group and the

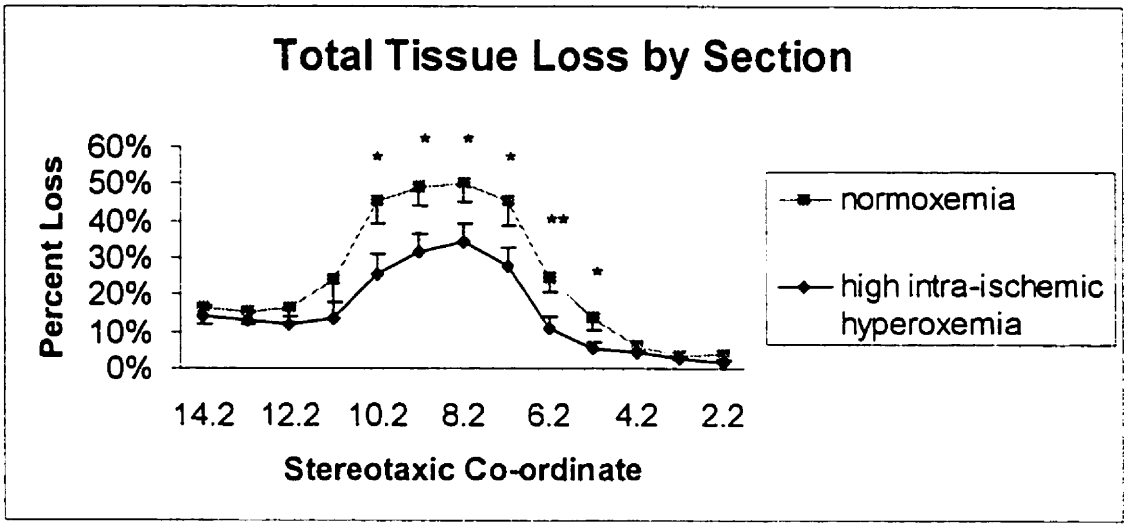

Figure 33 - Sectional analysis of total tissue loss for normoxemic and high intra-ischemic hyperoxemia groups.

control group occurred throughout the middle portion of the brain, at sections **10.2** (p = 0.0242), **9.2** (p = 0.0346), **8.2** (p = 0.0346), **7.2** (p = 0.0346), **6.2** (p = 0.0346), and **5.2** (p = 0.0167).

Reperfusion hyperoxemia (figure 34) also resulted in less overall damage when compared to the normoxemic group. In this group the most damage occurred at levels 10.2 to 7.2, averaging about 30% of the contralateral hemisphere section size. Significant differences between this treatment and the normoxemic treatment occurred at levels **10.2** (p = 0.0343), **9.2** (p = 0.0413), **8.2** (p = 0.0284), **6.2** (p = 0.0343), and **5.2** (p = 0.0082).

Consistent with all of the previous results, the rats that were exposed to hyperoxemia both during and after their stroke enjoyed the least amount of brain damage (figure 35). Damage is drastically reduced when compared to the normoxemic group, with the most damage, only about 17%, occurring at corresponding levels to the normoxemic group. Highly significant differences in total brain tissue lost between the intra-ischemic plus reperfusion hyperoxemia group and the normoxemic group occurred at levels **10.2** (p = 0.0009), **9.2** (p = 0.0009), **8.2** (p = 0.0003), **7.2** (p = 0.0007), **6.2** (p =

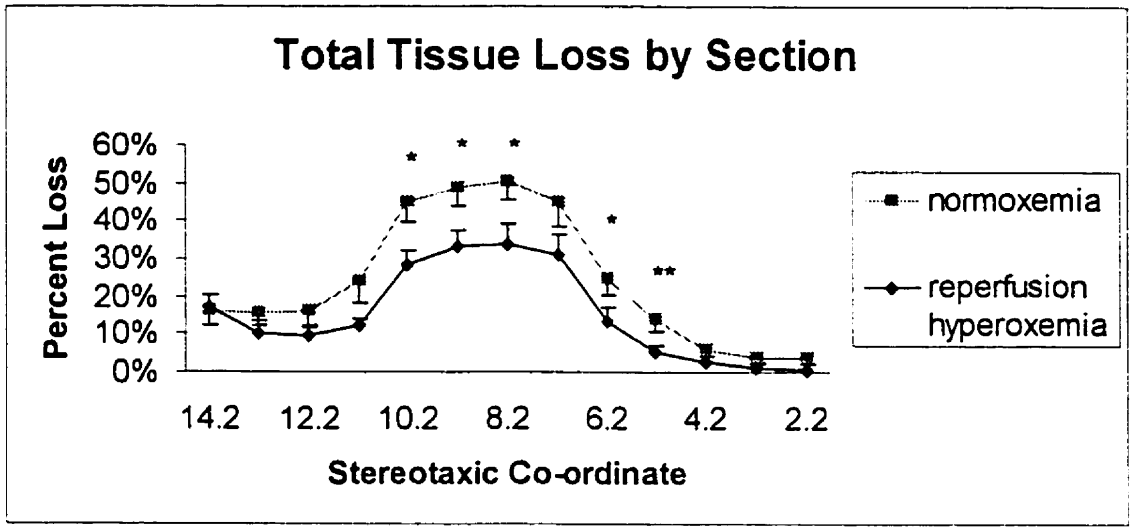

Figure 34 - Sectional analysis of total tissue loss for the normoxemic and reperfusion hyperoxemia groups.

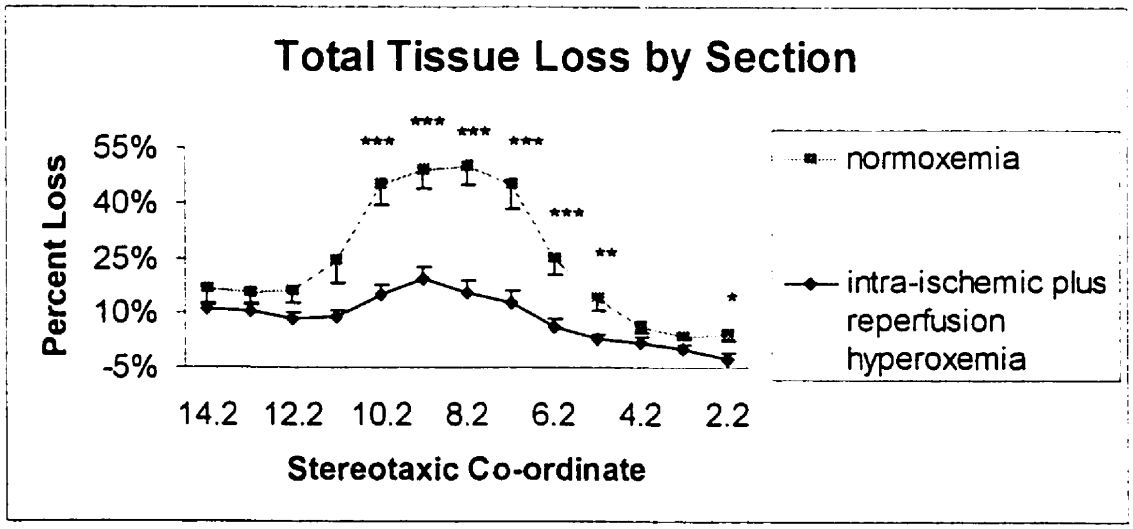

Figure 35 - Sectional analysis of total tissue loss for the normoxemic and intra-ischemic plus reperfusion hyperoxemia groups.

0.0007), **5.2** ($p = 0.0031$), and **2.2** ($p = 0.0137$). It is noteworthy that damage was less even occipitally, due to protection against atrophy (see above), and that this remote protection persisted even when total damage is considered.

Relationship Between PO_2 and Infarct Size

To examine whether there is a direct relationship between the level of hyperoxemia attained during the stroke and the size of the infarction, *i.e* to find out if a certain PO_2 results in a certain size of infarct, a regression analysis was done between intra-ischemic PO_2 and the size of infarcts for the normoxemic group and the two intra-ischemic hyperoxemia only groups. Results on all regressional analyses, including cortical damage, subcortical damage, total necrosis, atrophy, and total tissue loss on blood oxygen level were not statistically significant. This indicates that in this experiment, the level of blood oxygen is not a discrete indicator of infarct size – beneficial effects seen with intra-ischemic hyperoxemia seem to be an all-or-none phenomenon. To see if higher blood oxygen levels result in a smaller infarct size, a Spearman's Ranksum correlational test between PO_2 and infarct size was also done for the normoxic group and the two intra-ischemic hyperoxemia groups. Again the results of all correlational tests, including cortex, subcortex, total necrosis, atrophy, and total tissue loss to blood oxygen levels were not statistically significant, further indicating that the level of blood oxygen is not a discrete indicator of stroke size.

Correlation of Behavior and Histology

Both changes in behavior and the death of neurons are the result of cerebral ischemia. It is of utmost importance to determine whether the changes in behavior and the changes in brain structure are correlated, as neurologists use behavior to assess the neurological function of a presenting patient, yet histological analyses are not available unless neuroimaging is done, or until autopsy. After using a Spearman's Ranksum test to analyze whether a correlation exists between these two dependant variables, it was found

that in all cases the two variables were in fact correlated to a high level of significance. Cortical damage ($p = 0.0005$), subcortical damage ($p = 0.0023$), total necrosis ($p = 0.0009$), atrophy ($p < 0.0000$), and total tissue loss ($p < 0.0000$) were all significantly correlated to behavioral outcome on the Bederson Neurological scale.

CHAPTER FOUR: DISCUSSION

Conclusions for Treatments based on the Results

Low Levels of Intra-Ischemic Hyperoxemia

There is improvement in neurological outcome in all variables measured when the low intra-ischemic hyperoxemia group is compared to the normoxemic control group. Behaviorally, low levels of intra-ischemic hyperoxemia significantly improved Bederson neurological scores. As well, this treatment increased water consumption and weight gain, overall indicators of the health of the animal, although not to a statistically significant degree. Histologically, low levels of intra-ischemic hyperoxemia significantly reduced cortical damage, slightly reduced subcortical damage, slightly reduced the amount of atrophy, and significantly reduced the total tissue lost.

Based on these observations, it is concluded that, at least in the rat, low levels of intra-ischemic hyperoxemia provide neuroprotection against ischemic brain injury.

Since the behavioral results coincided with the histological results, it is also concluded that low levels of intra-ischemic hyperoxemia provide benefit at both the level of the synapse and the level of the neuron. It is impossible to decipher from this data, however, whether behavior is improved due to an effect on synapses exclusively, or whether the improvement in behavior is due to the salvaging of neurons.

High Levels of Intra-Ischemic Hyperoxemia

High levels of intra-ischemic hyperoxemia resulted in roughly the same amount of neuroprotection as the low intra-ischemic hyperoxemia treatment. Behavioral outcomes were improved with this treatment, resulting in significantly improved Bederson neurological scores. Water consumption and weight gain were increased with

this treatment, although not attaining statistical significance. There was also less brain damage with this treatment. The amount of cortical necrosis was reduced by a significant amount, subcortical damage was slightly reduced, and the amount of atrophy in the affected hemisphere was slightly reduced. A summary measure of brain damage, total tissue loss, was significantly reduced with high intra-ischemic hyperoxemia.

Based on these observations, it is concluded that high levels of intra-ischemic hyperoxemia, similar to low levels of intra-ischemic hyperoxemia, provide neuroprotection against ischemic damage, at least in the rat.

Also similar to low intra-ischemic hyperoxemia, the agreement between behavioral and histological outcomes indicates that high intra-ischemic hyperoxemia provides benefit to both the synapse and the entire neuron. Again, it is impossible to tell from these data alone whether the improvement in behavior was due to a synaptic effect independent of neuronal necrosis, or whether the synaptic benefit necessary for an improvement in behavior was directly related to the reduction of necrosis.

Proposed Mechanism of Neuroprotection with Intra-Ischemic Hyperoxemia

How could hyperoxemia during a stroke improve neurological outcome? As mentioned before, since hemoglobin is nearly 100% saturated with oxygen at normal PO_2 levels, significant gains cannot come from any increase in hemoglobin-bound oxygen. Instead, it is proposed that intra-ischemic hyperoxemia allows the freely dissolved oxygen in the blood serum to diffuse from vascularized neural tissue to ischemic tissue. This diffusion of molecular oxygen down the concentration gradient could sustain the oxygen concentration in ischemic tissue to a physiological amount, thereby maintaining the production of adequate levels of ATP. Neuroprotection with this treatment could

therefore arise from the maintenance of ischemic tissue ATP levels, thereby minimizing the production of free radical upon reperfusion, and reducing post-ischemic injury.

There were no statistical or practical differences in the degree of neuroprotection between the low level and high level of intra-ischemic hyperoxemia, as judged from both a regressional and correlational analysis. This suggests that, at least at normobaric levels, the beneficial effects of intra-ischemic hyperoxemia are an "all-or-none" phenomenon. Based on the present results, there seems to be no linear relationship between blood oxygen level and infarct size, nor is there a dose-response curve for blood oxygen level.

If freely dissolved oxygen does diffuse down the concentration gradient from vascularized areas to ischemic areas, it seems intuitive that higher levels of arterial hyperoxemia during a stroke would be more beneficial than lower levels of hyperoxemia. However, the results of the present experiments did not reveal this. How can this be reconciled? Ischemia may cause the ATP levels of the tissue to drop below a crucial "threshold" level, below which triggers the biochemical cascade that produces necrotizing free radicals upon reperfusion. The diffusion of oxygen to ischemic tissue during intra-ischemic hyperoxemia may prevent the ATP levels from dropping below this critical threshold level, preventing the production of reperfusion free radicals in an "all-or-none" fashion.

Reperfusion Hyperoxemia

This treatment consistently provided surprising results. Rather than impairing neurological outcome, as would have been predicted by the theory of reperfusion injury due to the generation of oxygen-derived free radicals, elevating the oxygen level in the blood that returns to ischemic tissue provided neuroprotection both behaviorally and, to a

lesser extent, histologically (see below). Rats in this treatment group displayed significantly improved Bederson neurological scores. They also drank more water and gained more weight, although these increases were not statistically significant. Although there was an improvement in all histological measurements, including cortical and subcortical necrosis, atrophy, and total tissue loss, none of the improvements were found to be statistically significant.

Based on these surprising results, it can be concluded that the decades old notion of reperfusion injury due to the generation of oxygen-derived free radicals may not be applicable to neural tissue. Increasing the substrate for the pathological free radicals, namely oxygen, should have increased damage according to that theory. However, increasing the potential for the generation of free radicals did not increase damage but rather *decreased* damage, sometimes to a statistically significant degree. It would be of interest to see if hyperoxemia augmented the production of free radicals in the reperfusion period.

It can also be concluded from group 4 that the reperfusion period is a potential time for stroke treatment. Reperfusion hyperoxemia was both safe and effective at reducing stroke size, at least in the rat species.

Proposed Mechanism of Neuroprotection with Reperfusion Hyperoxemia

How could these surprising results have been attained, since it is widely accepted that a large part of the injury from ischemia arises from the production of oxygen-derived free radicals upon reperfusion? How could increasing the substrate for free radical production decrease the amount of damage actually produced?

One possible explanation for the neuroprotective effects of reperfusion hyperoxemia involves the speed of oxygen diffusion. The speed with which a molecule travels down its concentration gradient is influenced by the absolute difference between the two concentrations. That is, diffusion will be more rapid between areas that are vastly different than areas which are closer in concentration. Hyperoxemic reperfusion may bring the ATP levels of the ischemic tissue back up to physiological levels quicker than normoxemic reperfusion due to the larger concentration gradient, and subsequent faster diffusion of oxygen into the ischemic tissue. This speed of diffusion may effectively shorten the period of free radical production, thereby reducing the amount of brain damage. Although the *absolute difference* in diffusion speeds may be extremely short, a matter of only milliseconds, the *proportion* of the period of free radical production may be decreased enough to result in significant neurological improvement. Similarly, hyperoxemic reperfusion may restore oxygen tension in the ischemic tissue faster than normoxemic reperfusion. If physiological tissue oxygen tension provides neuroprotection against free radicals which *are* produced upon reperfusion after normoxemic ischemia, the relatively rapid restoration of tissue oxygen tension with hyperoxemic reperfusion versus normoxemic may effectively reduce the period of time free radicals are damaging to the tissue, resulting in less overall damage.

Behavioral outcome was more favorable with this treatment than histological outcome. While Bederson neurological scores were significantly improved, histological results were only approaching significant improvement. How could reperfusion hyperoxemia produce (slightly) greater brain damage than the intra-ischemic hyperoxemia condition, yet provide the same behavioral improvement, since synapses

(and therefore behavior) are affected by the damage of a neuron? One possible way to reconcile this seemingly incompatible outcome is that ischemia may result in the inhibition of neural activity, producing alterations in behavior independent of any necrosis. The beneficial behavioral outcomes seen with both intra-ischemic hyperoxemia and reperfusion hyperoxemia may have arisen because both conditions reversed this neural inhibition, mitigating behavioral outcome. The improvement in behavior seen with reperfusion hyperoxemia may have resulted from a "fixing" of synaptic deficit that occurred even in those neurons which were not killed by the ischemia.

Intra-Ischemic and Reperfusion Hyperoxemia

Rats who experienced both intra-ischemic plus reperfusion hyperoxemia consistently had dramatic improvements in their neurological outcome. The degree of neuroprotection gained by this treatment was truly astonishing. Both behaviorally and histologically, the improvements seen when comparing this group to the control group were highly significant. Scores on the Bederson neurological scale were extremely reduced with this hyperoxemia treatment. Rats displayed an average score that approached no neurobehavioral deficit at all. They also drank significantly more water, and showed the greatest weight gain after the surgery. The histological analysis showed the same remarkable improvements with this treatment as behavioral analysis did. Cortical infarction was virtually eliminated. Subcortical damage, difficult to treat clinically and experimentally, was reduced by a highly significant degree. The volume of atrophy was reduced, similar to all other hyperoxemia treatments. Lastly, the total volume of tissue lost was reduced to an astonishing level with this intra-ischemic plus reperfusion hyperoxemia treatment. Low and high intra-ischemic hyperoxemia alone

produced total damages of 14% and 15% of the contralateral hemisphere, respectively, compared to a normoxemic total tissue loss of 25% of the contralateral hemisphere. The addition of reperfusion hyperoxemia to this treatment resulted in a total tissue loss of only 8% of the contralateral hemisphere.

It can be concluded based on these results that hyperoxemia initiated at the beginning of an ischemic period and maintained throughout the reperfusion period provides astronomical neuroprotection, at least in the rat. Because the results were impressive and free of any apparent side effects, it can be recommended that this treatment be subjected to clinical trial in stroke patients.

The concordance of the behavioral and histological outcomes suggests that this treatment provides synaptic protection, possibly in addition to the protection of the entire neuron. Again, it is impossible to decipher from this data whether behavior is improved due to an effect on synapses exclusively, or whether the improvement in behavior is due to the salvaging of neurons.

Proposed Mechanism of Neuroprotection with Intra-Ischemic plus Reperfusion Hyperoxemia

Since both intra-ischemic hyperoxemia and reperfusion hyperoxemia have advantageous outcomes, the astonishing success of the combination of these two treatments can be seen as a summation of the neuroprotection provided by each. Diffusion of oxygen into the ischemic tissue as a consequence of intra-ischemic hyperoxemia may prevent the drop in ATP levels of the tissue necessary for the production of free radicals upon reperfusion. Reperfusion hyperoxemia may be further beneficial by compensating for oxygen deprivation in ischemic tissue in ways intra-

ischemic hyperoxemia cannot. For example, the diffused oxygen from intra-ischemic hyperoxemia may be used up producing ATP in the tissue, eliminating the subsequent production of copious free radicals. Reperfusion hyperoxemia may then rapidly bring up oxygen tension of the ischemic tissue, reducing the amount of time that the oxygen tension in the tissue is dangerously low.

Neuroprotection in Specific Brain Regions

Neuroprotection in the Cortex

The relatively large beneficial effects of hyperoxemia in the cortex versus the subcortex could have arisen out of the overlapping of vasculature in this region. The marked reduction in cortical necrosis could have been a result of the anterior and posterior cerebral arteries "loading up" the territory of the middle cerebral artery with oxygen during and/or after the stroke. The collateral arteries could have either reduced the distance needed for the oxygen to diffuse during the stroke, or directly supplied the ischemic territory with increased oxygen through the blood upon reperfusion.

Neuroprotection in the Subcortex

As there is no collateral blood flow in this region, the subcortex is difficult to treat. Intra-ischemic plus reperfusion hyperoxemia significantly reduced damage in this region. The efficacy of this treatment in the subcortex despite of the lack of overlapping blood flow indicates its strong neuroprotective properties. The fact that there was not complete neuroprotection with this combination treatment subcortically, although there was almost complete cortical neuroprotection, supports the notion of oxygen diffusion to the ischemic tissue during ischemia. Oxygen would have to diffuse a greater distance from vascularized areas to reach the subcortex compared to the cortex, and for this reason

may have provided only a portion of the protection afforded in the cortex. Reperfusion hyperoxemia after hyperoxemic ischemia may have provided further neuroprotection in the subcortex, salvaging a significant amount of this tissue.

Atrophy

Tissue loss occurred not only in the areas that corresponded to necrosis, but also in areas anterior and posterior to the infarct. This indicates that in addition to macrophages cleaning up the dead tissue, trans-synaptic atrophy took place along the antero-posterior axis of the brain. Neurons which were damaged in the infarcted area would have degenerated even from areas which were spared from ischemia. For this reason, the entire ipsilateral hemisphere was affected by ischemia.

Because there were no differences between groups in the amount of atrophy, this suggests that the speed of the shrinkage of neural tissue after a stroke is not related to the overall amount of tissue necrosis. Cleaning up of dead tissue by macrophages and trans-synaptic atrophy seemed to take place at a constant pace, independent of the amount of tissue damage.

Possible Confounding Variables

Although the beneficial effects of arterial hyperoxemia during and after stroke may be due to factors directly related to oxygen and oxygen derived free radicals, it is also conceivable that the mitigating effects of hyperoxemia may be due to an indirect effect on other physiological parameters.

Temperature and Hyperoxemia

For example, it has been shown that at hyperbaric pressures, hyperoxia lowers the body temperature of experimental animals [109,110]. This has been interpreted as both a

toxic and an adaptive response mediated by the hypothalamic thermoregulartory center. As mentioned before, hypothermia has been shown to provide neuroprotection against ischemic injury[42]. This hyperbaric lowering of body temperature in animals has *not* been shown at normobaric pressure. Brain temperature in this experiment, as inferred by the ipsilateral temporalis muscle, was strictly controlled during the 60 minute ischemic period and the 60 minute monitored reperfusion period to normothermic levels. However, it is conceivable that the neuroprotective effects of hyperoxemia are indirect, caused by a reduction in body and/or brain temperature during the first days and nights after the stroke. It has been shown that perfusing neurons from the preoptic region of the hypothalamus, the area responsible for thermoregulation[111], with high levels of oxygen caused their thermosensitive properties to be lost, and further that the addition of a free radical scavenger prevented this loss of thermosensitivity[112]. This suggests that high oxygen and free radicals may reduce the ability of the body to regulate body temperature.

While the advantageous outcome with hyperoxemia may have been secondary to the lowering of body temperature, it is the opinion of this researcher that this was not the case. It was observed that animals given a hyperoxemic treatment were far more active than normoxemic rats, which moved very little in the first days following the stroke. Locomotor activity has been shown in animals to correlate with body temperature[113,114]. It is therefore unlikely that the beneficial effects of hyperoxemia are due to changes in temperature, since changes in temperature which did occur would correlate to activity levels, and would occur in the opposite direction to that expected to improve neurological outcome.

Cerebral Blood Flow and Hyperoxemia

It is also conceivable that the beneficial effects of hyperoxemia are due to a change in cerebral blood flow, thus decreasing the intensity of the ischemic insult for the hyperoxemia treatments. While hyperoxemia does in fact dilate blood vessels in the lungs, resulting in a greater blood flow [96], hyperoxemia causes the blood vessels of the brain to constrict, resulting in *less* blood flow [115,116]. Since the ischemic artery, by definition, is not exposed to this hyperoxemia, it does not constrict. The constriction of collateral arteries during hyperoxemia may create a "shunt" which forces more blood into the ischemic tissue, thus decreasing the ischemic bed. The beneficial effects of hyperoxemia may therefore be vascular, resulting from an increased amount of blood reaching the infarction.

Nitrous Oxide

In order to elevate blood oxygen levels, the fraction of inspired oxygen was increased to 100%. Since normal blood oxygen levels were attained by inhalation of 20% oxygen in an 80% nitrous oxide gas mixture, increasing the blood oxygen level also resulted in a decrease of nitrous oxide inhalation. The beneficial effects of hyperoxemia may have been secondary to this decrease of nitrous oxide inhalation. The effects of nitrous oxide on cerebral ischemia have been tested by comparing the neurological outcome of a fentanyl anesthesia/nitrogen inhalation control group to a fentanyl/nitrous oxide inhalation group[117]. The nitrous oxide group exhibited a significantly worse neurological outcome. This suggests that nitrous oxide may have a detrimental effect on cerebral ischemia. That study, unfortunately, had some problems which bring into question the validity of the results. First of all, the fentanyl/nitrogen control group

received twice as much of the fentanyl anesthetic as the fentanyl/nitrous oxide group. For this reason, it is impossible to determine whether the impaired neurological outcome was due to the inhalation of nitrous oxide, or to the smaller amount of fentanyl administered. As well, the pathological effects of nitrous oxide were suggested to be due to an increase in catecholamines. Other researchers, however, have found elevated levels of catecholamines to be in fact beneficial to ischemia[118]. Because of the confounding variable, and the inconsistent results between researchers, it can not be stated definitively that nitrous oxide is detrimental to stroke. It has also been shown that nitrous oxide in fact *does not* increase the size of infarct after middle cerebral artery in rats [119]. For these reasons it is unlikely that the beneficial effects of hyperoxemia on ischemia in this study are secondary to a reduction in nitrous oxide inhalation.

Nitrous oxide has also been shown to dilate cerebral blood vessels, thereby affecting cerebral blood flow. In healthy humans, 30% and 60% nitrous oxide inhalation in oxygen significantly increased blood flow when compared to a 100% oxygen control group[120]. Cerebral vasodilation during nitrous oxide inhalation has also been shown in the rat[121]. However, any vasodilatory effects of nitrous oxide would have been eliminated during hyperoxemia in this experiment. The normoxemic control group experienced nitrous oxide inhalation and therefore cerebral vasodilation, yet exhibited the greatest degree of damage after ischemia. This suggests that nitrous oxide mediated changes in blood flow are not responsible for the mitigating effects of hyperoxemia, since they work in the opposite direction to that expected to improve ischemic injury.

Halothane

Halothane, like many other anesthetics, has been shown to have a neuroprotective effect on ischemic injury[122-124]. In this study, the amount of halothane was held constant. Equal doses of halothane were used with equal experimental goals in mind, namely the holding of intra-ischemic blood pressure to 60 mm Hg. The beneficial effects of hyperoxemia were therefore not secondary to any anesthetic effect, since anesthesia levels were held constant throughout the experiment.

Limitations of this study – Application to the Real World

Although the results are encouraging for the treatment of ischemic injury, the present study is not a complete survey of the effects of hyperoxemia on stroke. It is tempting to take these results on the beneficial effects of hyperoxemia during and after stroke directly to the hospital to start testing whether this works in humans. But these results may be directly applicable only in the setting resembling the inflicted injury – a 60-minute stroke at a blood pressure of 60 mm Hg in male rats. These results indicate nothing about the effects of hyperoxemia and a stroke of longer duration. It cannot be concluded what the effects of hyperoxemia are on a stroke of 2 hours, 8 hours, or 24 hours. The blood pressure in this experiment, utilized to ensure consistent infarction between rats, is artificially low, as blood pressure spontaneously increases in response to cerebral ischemia. The hyperoxemia treatment may only work at this artificially reduced blood pressure level.

As mentioned before, there are fundamental differences between rodent and human brains that may prevent the results from being applicable across the animal kingdom. The size of a rat brain, about 2 grams, is obviously much smaller than the size

of a human brain, about 1300 g. If the maximum distance that oxygen can diffuse from vascularized areas is fixed, for example 1 mm, the significant neuroprotection displayed in rats may be lost in a larger brain. The preservation of a few cubic millimeters of neural tissue by means of oxygen diffusion from vascularized areas in a rat brain represents a substantial proportion of the total neural tissue. However, the preservation of the same few cubic millimeters of human neural tissue may not result in any statistical or, more importantly, functional significance.

Another relevant difference between rat and human brains is the relative differences in the diameter of the cerebral blood vessels. The diameter of the middle cerebral artery of a rat is about the same size as a small branch of a human middle cerebral artery cerebral artery. Fluid tends to flow through tubes in a streamlined fashion as though it is composed of a large number of concentric layers. This is called laminar flow [4]. Fluid near the wall of the tube moves the slowest since it experiences the greatest amount of resistance against the stationary wall. The rheology, or the flow of blood in the vessels, therefore differs between rats and humans. If the mitigating effects of hyperoxemia on ischemia are in any way related to the rate or amount of blood flowing in the vessels, the present results may only be applicable to the smaller diameter of the rat vasculature.

As mentioned before, due to the high density of neurons in rats, they have a higher basal cerebral metabolic rate than humans do. Hypermetabolic necrosis occurs when metabolism outstrips blood supply[125]. The metabolic rate for hypermetabolic necrosis is closer to rats than it is to the lower basal metabolic rate of humans. The beneficial effects of hyperoxemia may work in rats by altering the cerebral metabolic rate

to below the threshold for hypermetabolic necrosis. If this is the case, then these results may not be applicable to humans, since the hypermetabolic mechanism may not apply to human stroke.

Another thing that must be considered is that the rats in this study had their strokes under general anesthetic. Most humans who have a stroke are not under such anesthetics. Since it has been shown that anesthetics affect outcome from cerebral ischemia[122,123], the results of the present experiment may be dependant on this factor.

One other limitation of this study is that the Bederson neurological scale was given to the rats by the same experimenter who inflicted the stroke. Although every effort was made to be as objective as possible, the results would have been more valid if the Bederson neurological exam was given blindly, to ensure the elimination of any experimenter bias.

Behavior – Histology Correlation

It is desirable to improve the behavior of individuals who have had a stroke. While it has been suggested that histological investigation alone is not sufficient to properly assess the efficacy of a treatment[126], since infarct size may not correlate with behavioral outcome, in this particular study the correlation between the size of the infarct and the behavior exhibited was highly significant. This supports the claim made by Bederson et al[106] that the neurobehavioral assessment they devised is a reliable indicator of middle cerebral artery occlusion infarct size. In this study, an improvement in both neurobehavior and histological outcome was achieved with hyperoxemia. The improvement in both these measurements suggests that the experimental therapy is truly an effective one.

Future Research Directions

Oxygen Derived Free Radicals

Because the theory of oxygen-derived free radicals and ischemic injury is such a fundamental and longstanding hypothesis in stroke research, it is of utmost importance to know what the free radical correlates are for these completed experiments. If it is found that elevating blood oxygen levels improve behavioral and histological outcome, yet free radical levels are concurrently increased or unchanged, then the theory that oxygen-derived free radicals are damaging to neural tissue will be directly challenged.

It will be valuable to know what the free radical correlates are for each of the hyperoxemia treatments, as each may produce different free radical levels. Certain questions need to be answered: Is intra-ischemic hyperoxemia beneficial because the amount of free radicals produced upon reperfusion is actually decreased? Is there a correlation between the level of intra-ischemic PO_2 and the amount of free radicals produced? Does reperfusion hyperoxemia really result in an elevated production of free radicals? Is there a correlation between the PO_2 during reperfusion and the amount of free radicals produced? What is the effect on free radical production when intra-ischemic hyperoxemia is combined with reperfusion hyperoxemia?

Clinicians may be apprehensive about oxygen therapy for ischemic injury because of this theory of oxygen derived free radical production. Whether the concern is a valid one remains to be seen. The theory needs to be critically tested in combination with the effects of hyperoxemia on neurological outcome.

Tissue Oxygen Tension

In partnership with free radical level measurement, it would be valuable to know how the brain tissue oxygen levels are affected by hyperoxemia. Does intra-ischemic hyperoxemia maintain the oxygen level in the ischemic tissue to physiological levels, supporting the notion of freely dissolved oxygen diffusing from vascularized areas down the concentration gradient? Can the drop in ATP levels of the ischemic tissue necessary for the production of free radicals be avoided by maintaining tissue oxygen tension? Does normoxic or hyperoxemic tissue tension provide neuroprotection against otherwise damaging free radicals? How quickly is the oxygen tension in the tissue restored after hyperoxemic reperfusion versus normoxic reperfusion? Does the tension of oxygen in the tissue correlate with blood oxygen level?

Answering these questions will provide information about how hyperoxemia mitigates ischemic injury. It may lead into other concepts, which will further be able to improve neurological outcome by elevating oxygen levels.

Temperature

As brain temperature during and after a stroke has been shown to provide neuroprotection against ischemic injury, it is important to know if temperature has anything to do with the beneficial effects of hyperoxemia. Although the brain temperature was monitored during and directly after the stroke, it is necessary to know what happens to brain temperature in the first couple of days following the injury. Does intra-ischemic hyperoxemia result in a decrease of brain temperature after the stroke? If so, is hyperoxemia still beneficial if brain temperature is maintained to normothermia for days after the stroke? Is there a relationship between the level of blood oxygen and any

change in brain temperature? Does reperfusion hyperoxemia decrease brain temperature in the days following the stroke? Are the effects of reperfusion hyperoxemia still beneficial if brain temperature is maintained to normothermia after the stroke? What are the brain temperature correlates to a treatment of intra-ischemic plus reperfusion hyperoxemia? Is the large neuroprotection found with this treatment dependent on brain temperature?

Cerebral Blood Flow and Blood Pressure

Since hyperoxemia has been shown to decrease cerebral blood flow, it is important to verify what is happening to the blood flow of the brain with this treatment. Are the beneficial effects seen with intra-ischemic hyperoxemia maintained, or even increased, if blood flow during the stroke is controlled to match the normoxemic control group? Does increasing blood pressure, thereby increasing collateral blood flow, in combination with intra-ischemic hyperoxemia provide even greater neuroprotection? Are the beneficial effects of reperfusion hyperoxemia due to a decreased blood flow returning to the ischemic tissue? Are the benefits of reperfusion hyperoxemia maintained if blood flow is controlled to match normoxemic reperfusion? Does varying blood pressure during reperfusion hyperoxemia affect the neurological outcome? Are the drastic improvements seen with a combination of intra-ischemic and reperfusion hyperoxemia dependent on cerebral blood flow? Are these improvements influenced by blood pressure?

Nitrous Oxide

Because of a possible confound of a reduction of nitrous oxide inhalation during hyperoxemia, it is very important to verify that the beneficial effects seen with

hyperoxemia are not spuriously related to the anesthetic. It would therefore be valuable to repeat the above experiments with normal air, instead of nitrous oxide, as the gas utilized for the normoxemia condition. Decreasing the amount of ambient air inhaled, rather than decreasing the amount of nitrous oxide inhaled, is more representative of oxygen therapy for human stroke.

Delay of Hyperoxemia Onset

Another concept that needs to be clarified is how long after the beginning of a stroke is hyperoxemia beneficial. This is very important clinically, as many individuals may not realize that they are suffering a stroke, or be able to attain medical help, until many hours after the ischemia has begun.

Is intra-ischemic hyperoxemia still beneficial if it not initiated at the very beginning of a stroke? Are the benefits seen with reperfusion hyperoxemia still present if the ischemic tissue has already been adequately reperfused with normoxic blood? In other words, are the beneficial effects of reperfusion hyperoxemia dependent on a hyperoxic drenching of the formerly ischemic tissue?

Duration of Ischemia

As mentioned before, the stroke model in these experiments represent a restricted ischemic injury – a 1 hour stroke. It needs to be verified whether hyperoxemia is still beneficial with strokes of different duration. By halving, doubling, or tripling, the length of stroke, information can be gained about the relationship of hyperoxemia to the duration of ischemia. Is intra-ischemic hyperoxemia advantageous in the rat if the stroke length is decreased? Is intra-ischemic hyperoxemia still beneficial if the stroke length is increased? Is reperfusion hyperoxemia still beneficial if the duration of the stroke is

shorter or longer? Are the drastic improvements seen with intra-ischemic plus reperfusion hyperoxemia still present if the period of ischemia is varied?

Behavioral Observations

Several observations would merit further research. It seemed that reperfusion hyperoxemia resulted in a quicker recovery from surgery than normoxemic reperfusion did. The animals awoke from anesthesia and began moving around sooner with this treatment than normoxic reperfusion. As well, animals in the 24-hour oxygen rich environment seemed to be hyperactive when compared to the 24-hour normoxic recovery. It would be interesting and valuable to know whether these observations were indicative of a legitimate phenomenon. Additional behavioral testing of speed of recovery and activity levels after stroke in a hyperoxic environment is therefore suggested.

While not examined in this thesis, it would be interesting to know whether the variations in drinking and eating behavior were caused by the ischemic destruction and hyperoxemic salvation of certain brain regions. For example, osmoreceptors in the vascular organ of the lamina terminalis, the subfornical organ, and the anterior hypothalamus have been shown to be involved in the thirst response[127]. Although these structures are medial and therefore protected from ischemia in this stroke model, slight structural changes not discernable with eosin and hematoxlyin staining could have taken place here, such as alterations in the synapses, causing the variation in water consumption exhibited.

Bilateral lesions of the ventrolateral hypothalamus have been shown to cause aphagia (absence of eating), which can cause death by starvation[128]. While this structure

was also protected from ischemic necrosis in this model and any damage that did occur would have been unilateral, again slight structural changes in the synapses could have caused the variations in eating displayed by the animals.

Combination of Hyperoxemia with other Ischemia Treatment

As the infarct was not completely eliminated even with the intra-ischemic plus reperfusion hyperoxemia treatment, factors other than oxygen may contribute to the development of neuron death after ischemia. It is valuable to know what the effects of hyperoxemia are in combination with other infarct reducing agents, such as hypoglycemia, insulin, hypothermia, and, possibly, free radical scavengers. If hyperoxemia has adverse effects when combined with any of these factors, it would be useful to identify these and avoid needlessly exacerbating an injury.

Future Clinical Directions

The ultimate goal of any medical research is to improve the care level given to patients, and to improve the outcome for individuals with the prescribed treatment. The present research suggests that individuals who have suffered a stroke should be placed in a clinical trial of oxygen therapy, either through an oxygen mask, in an oxygen tent, or, more effectively, while intubated. It is important that a clinical trial be undertaken to ensure that the beneficial effects seen with rats is not species specific. It should be assessed whether high blood oxygen levels improve behavioral and the amount of neuronal death in humans as it does in rats. Neuroimaging techniques such as PET scans and/or MRI images could be used to assess the size of brain damage, in addition to neurologic scoring. If clinical trials show that oxygen therapy is indeed beneficial for stroke victims, the impact on the medical community will be high, as those who have had

a stroke or who are at risk for one will have an inexpensive and easy treatment modality to improve their ultimate outcome.

REFERENCES:

1. Nolte J. Blood Supply of the Brain. The Human Brain: An Introduction to its Functional Anatomy. 3rd ed., Mosby-Year Book, Inc., St. Louis, MO. 1993; pp 76-100

2. Martin JH. Vasculature of the Central Nervous System and the Cerebrospinal Fluid. Neuroanatomy Text and Atlas. 2nd ed., Appleton & Lange, Stamford, CT. 1996; pp 93-121

3. Auer RN, Benveniste H. Hypoxia and related conditions. In: Graham D, Lantos P (eds). Greenfield's Neuropathology. 6th ed., Vol. 1. Edward Arnold, London. 1997; pp 263-314

4. Seeley RR, Stephens TD, Tate P. Cardiovascular System: Peripheral Circulation and Regulation. Anatomy & Physiology. 2nd ed., Mosby - Year Book, Inc., St. Louis, MO. 1992; pp 645-697

5. Garcia JM, Anderson, ML. Circulatory Disorders and their Effects on the Brain. In: Davis RL, Roberston DM (eds). Textbook of Neuropathology. 2nd ed., Williams and Wilkens, Baltimore, MD. 1991; pp 621-718

6. Deutch AY & Roth RH. Neurotransmitters. In: Zigmond MJ, Bloom FE, Landis SC, Roberts JL, Squire LR (eds). Fundamental Neuroscience. Academic Press, San Diego, CA. 1999; pp 193-234

7. Olney JW. Glutamate-induced retinal degeneration in neonatal mice. Electron microscopy of the acutely evolving lesions. J Neuropathol Exp Neurol 1969;28:455-474

8. Olney JW. Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. Science 1969;164:719-721

9. Olney JW. Glutamate-induced neuronal necrosis in the infant mouse hypothalamus. J Neuropathol Exp Neurol 1971;30:75-90

10. Benveniste H. The excitotoxin hypothesis in relation to cerebral ischemia. Cerebrovasc Brain Metab Rev 1991;3:213-245

11. Benveniste H, Drejer J, Schousboe A, Diemer NH. Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. J Neurochem 1984;43:1369-1374

12. Bekkers JM, Stevens CF. NMDA and non-NMDA receptors are co-localized at individual excitatory synapses in cultured rat hippocampus. Nature 1989;341:230-233

13. Nakanishi S. Molecular diversity of glutamate receptors and implications for brain function. Science 1992;258:597-603

14. Simon RP, Swan JH, Griffiths T, Meldrum BS. Blockade of N-methyl-D-aspartate receptors may protect against ischemic damage in the brain. Science 1984;226:850-852

15. Buchan AM, Xue D, Huang ZG, Smith KH, Lesiuk H. Delayed AMPA receptor blockade reduces cerebral infarction induced by focal ischemia. Neuroreport 1991;2:473-476

16. Bruno V, Battaglia G, Kingston A, O'Neill MJ, Catania MV, Di Grezia R, Nicoletti F. Neuroprotective activity of the potent and selective mGlu1a metabotropic glutamate receptor antagonist, (+)-2-methyl-4 carboxyphenylglycine (LY367385): comparison with LY357366, a broader spectrum antagonist with equal affinity for mGlu1a and mGlu5 r. Neuropharmacology 1999;38:199-207

17. Nurse S, Corbett D. Neuroprotection after several days of mild, drug-induced hypothermia. J Cereb Blood Flow Metab 1996;16:474-480

18. Buchan AM, Li H, Cho S, Pulsinelli WA. Blockade of the AMPA receptor prevents CA1 hippocampal injury following severe but transient forebrain ischemia in adult rats. Neurosci Lett 1991;132:255-258

19. Zivin JA, Fisher M, DeGirolami U, Hemenway CC, Stashak J. Tissue plasminogen activator reduces neurological damage after cerebral embolism. Science 1985;230:1289-1292

20. Seeley RR, Stephens TD, Tate P. Cardiovascular System: Blood. Anatomy & Physiology. 2nd ed., Mosby - Year Book, Inc., St. Louis, MO. 1992; pp 585-609

21. Marler J, et al; for: The National Institute of Neurological Disorders and Stroke t-PA Stroke Study Group. Tissue plasminogen activator for acute ischemic stroke. N Engl J Med 1995;333:1581-1587

22. Bigelow WG, Callaghan JC, Hopps JA. General hypothermia for experimental intracardiac surgery. Ann Surg 1950;132:531-537

23. Pontius RG, Bloodwell RD, Cooley DA, De Bakey ME. The use of hypothermia in the prevention of brain damage following temporary arrest of cerebral circulation: experimental observations. Surg Forum 1954;5:224-228

24. Rosomoff HL. Hypothermia and cerebral vascular lesions. I. Experimental interruption of the middle cerebral artery during hypothermia. J Neurosurg 1956;13:244-255

25. Rosomoff HL. Hypothermia and cerebral vascular lesions. II. Experimental middle cerebral artery interruption followed by induction of hypothermia. Arch Neurol Psychiat (Chic) 1957;78:454-464

26. Busto R, Dietrich WD, Globus MY-T, Valdés I, Scheinberg P, Ginsberg MD. Small differences in intraischemic brain temperature critically determine the extent of ischemic neuronal injury. J Cereb Blood Flow Metab 1987;7:729-738

27. Minamisawa H, Nordström C-H, Smith M-L, Siesjö BK. The influence of mild body and brain hypothermia on ischemic brain damage. J Cereb Blood Flow Metab 1990;10:365-374

28. Green EJ, Dietrich WD, van Dijk F, Busto R, Markgraf CG, McCabe PM, Ginsberg MD, Schneiderman N. Protective effects of brain hypothermia on behavior and histopathology following global cerebral ischemia in rats. Brain Res 1992;580:197-204

29. Dietrich WD, Busto R, Alonso O, Globus MY-T, Ginsberg MD. Intraischemic but not postischemic brain hypothermia protects chronically following global forebrain ischemia in rats. J Cereb Blood Flow Metab 1993;13:541-549

30. Nurse S, Corbett D. Direct measurement of brain temperature during and after intraischemic hypothermia: correlation with behavioral, physiological, and histological endpoints. J Neurosci 1994;14:7726-7734

31. Onesti ST, Baker CJ, Sun PP, Solomon RA. Transient hypothermia reduces focal ischemic brain injury in the rat. Neurosurgery 1991;29:369-373

32. Baker CJ, Onesti ST, Solomon RA. Reduction by delayed hypothermia of cerebral infarction following middle cerebral artery occlusion in the rat: a time-course study. J Neurosurg 1992;77:438-444

33. Chen H, Chopp M, Zhang ZG, Garcia JH. The effect of hypothermia on transient middle cerebral artery occlusion in the rat. J Cereb Blood Flow Metab 1992;12:621-628

34. Xue D, Huang Z-G, Smith KE, Buchan AM. Immediate or delayed mild hypothermia prevents focal cerebral infarction. Brain Res 1992;587:66-72

35. Busto R, Dietrich WD, Globus MY-T, Ginsberg MD. Postischemic moderate hypothermia inhibits CA1 hippocampal ischemic neuronal injury. Neurosci Lett 1989;101:299-304

36. Boris-Möller F, Smith M-L, Siesjö BK. Effect of hypothermia on ischemic brain damage: a comparison between preischemic and postischemic cooling. Neurosci Res Comm 1989;5:87-94

37. Chopp M, Chen H, Dereski MO, Garcia JH. Mild hypothermic intervention after graded ischemic stress in rats. Stroke 1991;22:37-43

38. Welsh FA, Harris VA. Postischemic hypothermia fails to reduce ischemic injury in gerbil hippocampus. J Cereb Blood Flow Metab 1991;11:617-620

39. Chen H, Chopp M, Vande Linde AM, Dereski MO, Garcia JH, Welch KM. The effects of post-ischemic hypothermia on the neuronal injury and brain metabolism after forebrain ischemia in the rat. J Neurol Sci 1992;107:191-198

40. Colbourne F, Corbett D. Delayed and prolonged post-ischemic hypothermia is neuroprotective in the gerbil. Brain Res 1994;654:265-272

41. Colbourne F, Corbett D. Delayed postischemic hypothermia: a six month survival study using behavioral and histologic assessments of neuroprotection. J Neurosci 1995;15:7250-7260

42. Colbourne F, Sutherland G, Corbett D. Postischemic hypothermia. A critical appraisal with implications for clinical treatment. Mol Neurobiol 1997;14:55-85

43. Dietrich WD, Busto R, Valdes I, Loor Y. Effects of normothermic versus mild hyperthermic forebrain ischemia in rats. Stroke 1990;21:1318-1325

44. Minamisawa H, Smith M-L, Siesjö BK. The effect of mild hyperthermia and hypothermia on brain damage following 5, 10, and 15 minutes of forebrain ischemia. Ann Neurol 1990;28:26-33

45. Sutherland GR, Lesiuk H, Hazendonk P, Peeling J, Buist R, Kozlowski P, Jazinski A, Saunders JK. Magnetic resonance imaging and 31P magnetic resonance spectroscopy study of the effect of temperature on ischemic brain injury. Can J Neurol Sci 1992;19:317-325

46. Chen H, Chopp M, Welch KM. Effect of mild hyperthermia on the ischemic infarct volume after middle cerebral artery occlusion in the rat. Neurology 1991;41:1133-1135

47. Baena RC, Busto R, Dietrich WD, Globus MY-T, Ginsberg MD. Hyperthermia delayed by 24 hours aggravates neuronal damage in rat hippocampus following global ischemia. Neurology 1997;48:768-773

48. Chopp M, Chen H, Ho K-L, Dereski MO, Brown E, Hetzel FW, Welch KMA. Transient hyperthermia protects against subsequent forebrain ischemic cell damage in the rat. Neurology 1989;39:1396-1398

49. Li PA, Shamloo M, Smith M-L, Katsura K, Siesjö BK. The influence of plasma glucose concentrations on ischemic brain damage is a threshold function. Neurosci Lett 1994;177:63-65

50. Yip PK, He YY, Hsu CY, Garg N, Marangos P, Hogan EL. Effect of plasma glucose on infarct size in focal cerebral ischemia-reperfusion. Neurology 1991;41:899-905

51. Candelise L, Landi G, Orazio EN, Boccardi E. Prognostic significance of hyperglycemia in acute stroke. Arch Neurol 1985;42:661-663

52. Drummond JC, Moore SS. The influence of dextrose administration on neurologic outcome after temporary spinal cord ischemia in the rabbit. Anesthesiology 1989;70:64-70

53. Folbergrová J, Memezawa H, Smith M-L, Siesjö BK. Focal and perifocal changes in tissue energy state during middle cerebral artery occlusion in normo- and hyperglycemic rats. J Cereb Blood Flow Metab 1992;12:25-33

54. Marie C, Bralet AM, Gueldry S, Bralet J. Fasting prior to transient cerebral ischemia reduces delayed neuronal necrosis. Metab Brain Dis 1990;5:65-75

55. Yager JY, Heitjan DF, Towfighi J, Vannucci RC. Effect of insulin-induced and fasting hypoglycemia on perinatal hypoxic-ischemic brain damage. Pediatr Res 1992;31:138-142

56. Seeley RR, Stephens TD, Tate P. Endocrine Glands. Anatomy & Physiology. 2nd ed., Mosby - Year Book, Inc., St. Louis, MO. 1992; pp 545-581

57. Voll CL, Auer RN. The effect of post-ischemic blood glucose levels on the density and distribution of ischemic brain damage in the rat. Ann Neurol 1988;24:638-646

58. Voll CL, Auer RN. Insulin attenuates ischemic brain damage independent of its hypoglycemic effect. J Cereb Blood Flow Metab 1991;11:1006-1014

59. Zhu CZ, Auer RN. Intraventricular administration of insulin and IGF-1 in transient forebrain ischemia. J Cereb Blood Flow Metab 1994;14:237-242

60. Hamilton MG, Tranmer BI, Auer RN. Insulin reduction of cerebral infarction due to transient focal ischemia. J Neurosurg 1995;82:262-268

61. Zhu CZ, Auer RN. Graded hypotension and MCA occlusion duration: effect in transient focal ischemia. J Cereb Blood Flow Metab 1995;15:980-988

62. Drummond JC, OH Y-S, Cole DJ, Shapiro HM. Phenylephrine-induced hypertension reduces ischemia following middle cerebral artery occlusion in rats. Stroke 1989;10:1538-1544

63. Hayashi S, Nehls DG, Kieck CF, Vielma J, DeGirolami U, Crowell RM. Beneficial effects of induced hypertension on experimental stroke in awake monkeys. J Neurosurg 1984;60:151-157

64. Guyton AC. Acute hypertension in dogs with cerebral ischemia. Am J Physiol 1948;154:45-54

65. Kogure K, Busto R, Scheinberg P. The role of hydrostatic pressure in ischemic brain edema. Ann Neurol 1981;9:273-282

66. Fisher CM. Pathological observations in hypertensive cerebral hemorrhage. J Neuropathol Exp Neurol 1971;30:536-550

67. Steen PA, Michenfelder JD, Milde JH. Incomplete versus complete cerebral ischemia: Improved outcome with a minimal blood flow. Ann Neurol 1979;6:389-398

68. Goldman MS, Anderson RE, Meyer FB. Effects of intermittent reperfusion during temporary focal ischemia. J Neurosurg 1992;77:911-916

69. Simon RP. Hypoxia versus ischemia. Neurology 1999;52:7-8

70. Miyamoto O, Auer RN. Hypoxia, hyperoxia, ischemia and brain necrosis. Neurology 2000;54:362-371

71. Kogure K, Scheinberg P, Fujishima M, Busto R, Reinmuth OM. Effects of hypoxia on cerebral autoregulation. Am J Physiol 1970;219:1393-1396

72. Jason GW, Pajurkova EM, Lee RG. High-altitude mountaineering and brain function: Neuropsychological testing of members of a Mount Everest expedition. Aviat Space Environ Med 1989;60:170-173

73. McCord JM. Oxygen-derived free radicals in postischemic tissue injury. N Engl J Med 1985;312:159-163

74. Mizoi K, Suzuki J, Imaizumi S, Yoshimoto T. Development of new cerebral protective agents: the free radical scavengers. Neurol Res 1986;8:75-80

75. Kontos HA, Wei EP. Cerebral arteriolar damage by arachidonic acid and prostaglandin G2. Science 1980;209:1242-1244

76. Aktan S, Aykut C, Oktay S, Yegen B, Keles E, Aykac I, Ercan S. The alterations of leukotriene C4 and prostaglandin E2 levels following different ischemic periods in rat brain tissue. Prostagland Leukot Essent Fatty Acids 1991;42:67-71

77. Suzuki J, Abiko H, Mizoi K, Oba M, Yoshimoto T. Protective effect of phenytoin and its enhanced action by combined administration with mannitol and vitamin E in cerebral ischaemia. Acta Neurochir (Wien) 1987;88:56-64

78. Wei EP, Kontos HA, Dietrich WD, Povlishock JT, Ellis EF. Inhibition by free radical scavengers and by cyclooxygenase inhibitors of pial arteriolar abnormalities from concussive brain injury in cats. Circ Res 1981;48:95-103

79. Thordstein M, Bagenholm R, Thiringer K, Kjellmer I. Scavengers of free oxygen radicals in combination with magnesium ameliorate perinatal hypoxic-ischemic brain damage in the rat. Pediatr Res 1993;34:23-26

80. Liu TH, Beckman JS, Freeman BA, Hogan EL, Hsu CY. Polyethylene glycol-conjugated superoxide dismutase and catalase reduce ischemic brain injury. Am J Physiol 1989;256:H589-593

81. Sakamoto A, Ohnishi ST, Ohnishi T, Ogawa R. Relationship between free radical production and lipid peroxidation during ischemia-reperfusion injury in the rat brain. Brain Res 1991;554:186-192

82. Tan S, Zhou F, Nielsen VG, Wang Z, Gladson CL, Parks DA. Increased injury following intermittent fetal hypoxia-reoxygenation is associated with increased free radical production in fetal rabbit brain. J Neuropathol Exp Neurol 1999;58:972-981

83. Zhao W, Richardson JS, Mombourquette MJ, Weil JA, Ijaz S, Shuaib A. Neuroprotective effects of hypothermia and U-78517F in cerebral ischemia are due to reducing oxygen-based free radicals: an electron paramagnetic resonance study with gerbils. J Neurosci Res 1996;45:282-288

84. Ulatowski JA, Kirsch JR, Traystman RJ. Hypoxic reperfusion after ischemia in swine does not improve acute brain recovery. Am J Physiol 1994;267:H1880-1887

85. Zwemer CF, Whitesall SE, D'Alecy LG. Hypoxic cardiopulmonary-cerebral resuscitation fails to improve neurological outcome following cardiac arrest in dogs. Resuscitation 1995;29:225-236

86. Lipinski CA, Hicks SD, Callaway CW. Normoxic ventilation during resuscitation and outcome from asphyxial cardiac arrest in rats. Resuscitation 1999;42:221-229

87. Agardh C-D, Zhang H, Smith M-L, Siesjö BK. Free radical production and ischemic brain damage: influence of postischemic oxygen tension. Int J Dev Neurosci 1991;9:127-138

88. Donald KW. Oxygen poisoning in man. Part I. Br Med J 1947;1:667-672

89. Balentine JD. Oxygen Intolerance. Pathology of Oxygen Toxicity. Academic Press, New York, NY. 1982; pp 12-30

90. Balentine JD, Gutsche BB. Central nervous system lesions in rats exposed to oxygen at high pressure. Am J Pathol 1966;48:107-127

91. Behnke AR, Johnson FS, Poppen JR, Motley EP. The effects of oxygen on man at pressures from one to four atmospheres. Am J Physiol 1935;110:565-572

92. Knight JA. Free radicals: their history and current status in aging and disease. Ann Clin Lab Sci 1998;28:331-346

93. Ahdab-Barmada M, Moossy J, Painter M. Pontosubicular necrosis and hyperoxemia. Pediatrics 1980;66:840-847

94. Ahdab-Barmada M, Moossy J, Nemoto EM, Lin MR. Hyperoxia produces neuronal necrosis in the rat. J Neuropathol Exp Neurol 1986;45:233-246

95. Meijne NG. Hyperbaric Oxygenation: Physical and Physiological Aspects. Hyperbaric Oxygen and its Clinical Value: With Special Emphasis on Biochemical and Cardiovascular. Charles C. Thomas, Springfield, Ill. 1970; pp 14-18

96. Seeley RR, Stephens TD, Tate P. Respiratory System. Anatomy & Physiology. 2nd ed., Mosby - Year Book, Inc., St. Louis, MO. 1992; pp 735-769

97. Cherniack RM, Cherniack L, Naimark A. Oxygen and Carbon Dioxide Exchanges Between Gas and Blood. Respiration in Health and Disease. 2nd ed., W.B. Saunders Company, Philadelphia, PA. 1972; pp 70-102

98. Ranney HM, Sharma V. Structure and Function of Hemoglobin. In: Beutler E, Lichtman MA, Coller BS, Kipps TJ (eds). Williams Hematology. 5th ed., McGraw-Hill, Inc., New York, NY. 1995; pp 417-425

99. Telen MJ. The Mature Erythrocyte. In: Lee GR, Bithell TC, Foerster J, Athens JW, Lukens JN (eds). Wintrobe's Clinical Hematology. 9th ed., Vol. 1. Lea & Febiger, Philadelphia, PA. 1993; pp 101-133

100. Steinberg MH, Benz EJ. Hemoglobin Synthesis, Structure and Function. In: Hoffman R, Benz EJ, Shattil SJ, Furie B, Cohen HJ (eds). Hematology: Basic Principles and Practice. Churchill Livingstone, Inc., New York, NY. 1991; pp 291-302

101. Balentine JD. Principles of Hyperoxic Pathophysiology. Pathology of Oxygen Toxicity. Academic Press, New York, NY. 1982; pp 31-34

102. Diamond MC, Scheibel AB, Elson LM. Support Systems of the Central Nervous System. The Human Brain Coloring Book. HarperCollins Publishers, Inc., New York, NY. 1985; pp 9:3-9:5

103. Longa EZ, Weinstein PR, Carlson S, Cummins R. Reversible middle cerebral artery occlusion without craniectomy in rats. Stroke 1989;20:84-91

104. Coyle P. Arterial patterns of the rat rhinencephalon and related structures. Exp Neurol 1975;49:671-690

105. Koizumi J, Yoshida Y, Nakazawa T, Ooneda G. Experimental studies of ischemic brain edema 1. A new experimental model of cerebral embolism in rats in which recirculation can be introduced in the ischemic area. Jpn J Stroke 1986;8:1-8

106. Bederson JB, Pitts LH, Tsuji M, Nishimura MC, Davis RL, Bartkowski H. Rat middle cerebral artery occlusion: evaluation of the model and development of a neurologic examination. Stroke 1986;17:472-476

107. Kolb B, Whishaw IQ. Recovery of Function. Fundamentals of Human Neuropsychology. 4th ed., W.H. Freeman and Company, New York, NY. 1996; pp 539-565

108. Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates. 2nd ed., Academic Press, Inc., San Diego, CA. 1986

109. Bert P. In: Translated into English by Hitchcock MA & Hitchcock FA (eds). La Pression Barometrique. Recherches de Physiologie Experimentale.. College Book Co., Columbus, OH. 1878

110. Puglia CD, Glauser EM, Glauser SC. Core temperature response of rats during exposure to oxygen at high pressure. J Appl Physiol 1974;36:149-153

111. Card JP, Swanson LW, Moore RY. The Hypothalamus: An Overview of Regulatory Systems. In: Zigmond MJ, Bloom FE, Landis SC, Roberts JL, Squire LR (eds). Fundamental Neuroscience. Academic Press, San Diego, CA. 1999; pp 1013-1026

112. Shibata M, Blatteis CM. High perfusate PO2 impairs thermosensitivity of hypothalamic thermosensitive neurons in slice preparations. Brain Res Bull 1991;26:467-471

113. Weinert D, Waterhouse J. Diurnally changing effects of locomotor activity on body temperature in laboratory mice. Physiol Behav 1998;63:837-843

114. Buttner D, Wollnik F. Spontaneous short-term fluctuations in the daily pattern of heart rate, body temperature and locomotor activity in the laboratory rat. Lab Anim 1982;16:319-326

115. Sjöberg F, Gustafsson U, Eintrei C. Specific blood flow reducing effects of hyperoxaemia on high flow capillaries in the pig brain. Acta Physiol Scand 1999;165:33-38

116. Omae T, Ibayashi S, Kusuda K, Nakamura H, Yagi H, Fujishima M. Effects of high atmospheric pressure and oxygen on middle cerebral blood flow velocity in humans measured by transcranial Doppler. Stroke 1998;29:94-97

117. Hoffman WE, Baughman VL, Albrecht RF. Interaction of catecholamines and nitrous oxide ventilation during incomplete brain ischemia in rats. Anesth Analg 1993;77:908-912

118. Koide T, Wieloch TW, Siesjö BK. Circulating catecholamines modulate ischemic brain damage. J Cereb Blood Flow Metab. 1986;6:559-565

119. Warner DS, Zhou JG, Ramani R, Todd MM, McAllister A. Nitrous oxide does not alter infarct volume in rats undergoing reversible middle cerebral artery occlusion. Anesthesiology 1990;73:686-693

120. Field LM, Dorrance DE, Krzeminska EK, Barsoum LZ. Effect of nitrous oxide on cerebral blood flow in normal humans. Br J Anaesth 1993;70:154-159

121. Carlsson C, Vasthare US, Tuma RF, Rocco MJ, Dombkoski RA. Influence of nitrous oxide administration and discontinuation thereof on blood flow in cerebral cortex, cerebellum and brain stem in the rat. Acta Anaesthesiol Scand 1991;35:771-775

122. Saito R, Graf R, Hubel K, Fujita T, Rosner G, Heiss WD. Reduction of infarct volume by halothane: effect on cerebral blood flow or perifocal spreading depression-like depolarizations. J Cereb Blood Flow Metab 1997;17:857-864

123. Warner DS, Ludwig PS, Pearlstein R, Brinkhous AD. Halothane reduces focal ischemic injury in the rat when brain temperature is controlled. Anesthesiology 1995;82:1237-1245

124. Kurihara J, Tomita H, Ochiai N, Kato H. Protection by halothane of the vagal baroreflex system from transient global cerebral ischemia in dogs. Jpn J Pharmacol 1992;60:63-66

125. Auer RN. Points of view. Early application of the results of animal experimentation to clinical trials: Con. J Neurosurg Anesthesiol 1996;8:73-77

126. Corbett D, Nurse S. The problem of assessing effective neuroprotection in experimental cerebral ischemia. Prog Neurobiol 1998;54:531-548

127. Stricker EM, Verbalis JG. Water Intake and Body Fluids. In: Zigmond MJ, Bloom FE, Landis SC, Roberts JL, Squire LR (eds). Fundamental Neuroscience. Academic Press, San Diego, CA. 1999; pp 1111-1126

128. Anand BK, Brobeck JR. Hypothalamic control of food intake in rats and cats. Yale Journal of Biological Medicine 1951;24:123-140