1994

Use of cortical grafts in hypoxic-ischemic brain injury in neonatal rats

M. Hany Elsayed
Loyola University Chicago

LOYOLA UNIVERSITY OF CHICAGO

USE OF CORTICAL GRAFTS IN HYPOXIC-ISCHEMIC BRAIN INJURY IN NEONATAL RATS

A THESIS SUBMITTED TO

THE FACULTY OF THE GRADUATE SCHOOL

IN CANDIDACY FOR THE DEGREE OF

MASTER OF SCIENCE

PROGRAM IN NEUROSCIENCE

BY

M. HANY ELSAYED

CHICAGO, ILLINOIS

MAY, 1994

DEDICATION

To the memory of my father, Fathy Ahmed Elsayed, whose life lives on in my mind and serves as a constant example of hard work, honesty and dedication. I thank you, Dad, for enabling me to learn and succeed.

ACKNOWLEDGMENTS

I sincerely thank Dr. Anthony J. Castro, my thesis advisor and mentor, for his expert scientific advice, creative ideas, and for giving me a chance to complete this matter successfully. I thank the members of my thesis committee, Drs. Edward J. Neafsey and Thomas F. Myers for their time, support and constructive criticism especially Dr. Neafsey, it was not in vain.

I am especially grateful to Thomas Hogan and Pamela Shaw for their technical assistance with this project. I also thank the members and staff of the Department of Cell Biology, Neurobiology and Anatomy at Loyola for their friendly treatment.

Finally and foremost I thank our creator, for bringing us to this earth to learn what we would have never learned. Thank you God for giving me the opportunity and the strength to learn some.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AChE	Acetylcholinesterase
AD	Anterodorsal
AV	Anteroventral
B	Brain
CA	Cornu ammonis
CBF	Cerebral Blood Flow
CCA	Common Carotid Artery
CNS	Central Nervous System
CPu	Caudate-Putamen
ctx	Cortex
DLG	Dorsolateral geniculate
E	Embryonic
g	Gyrus
HI	Hypoxia-Ischemia
HIE	Hypoxia-Ischemic Encephalopathy
LP	Lateral posterior
MCA	Middle Cerebral Artery
MHb	Medial habenular nucleus

NMDA	N-Methyl-D-Aspartate
PT	Parataenial
RT	Reticular
Rfc	Right frontal cortex
Tp	Transplant
V.dil	Ventricular dilatation
VL	Ventrolateral
VPL	Ventral posterolateral
VPM	Ventral posteromedial

CHAPTER I

INTRODUCTION

Brain damage due to hypoxia-ischemia (HI) during early development remains a major cause of neurological disorder arising in the perinatal period, and subsequent sequelae include mental retardation, learning difficulty, and cerebral palsy. These various sequelae are due to lesions involving different areas of the brain. In the case of cerebral palsy, it is the motor cortical area and the basal ganglia that are typically damaged. As there is no available clinical means to restore function of neurons damaged by HI insult, the management of this disorder is typically supportive treatment to minimize brain injury and rehabilitative treatment to reduce the impact of this brain injury on retarded functions.

Using a newborn rodent model of HI brain damage, several studies have examined various methods used to reduce the neuronal damage induced by HI at birth (Barks et al., 1991; Ford et al., 1989; Hattori and Wasterlian, 1990; McDonald et al., 1987). Based on reports demonstrating cytopathological changes within hours of the HI insult, with advanced ischemic cell changes being found by 24 hours (Ikonomidou et al., 1989; Rice et al., 1981), these experiments invariably involved treatments administered immediately before, after or during the HI insult. In contrast to these therapeutic strategies, the purposes of this study are to: 1) establish a neonatal rat

model of hypoxia-ischemia that produces a predictable, well-defined brain lesion, 2) examine the survival of fetal cortical tissue transplanted into the area of the hypoxic-ischemic brain lesion several days after the insult, 3) study the growth and appearance of the graft and its effect on surrounding structures at different time intervals after transplantation to see if graft would replace damaged neurons and if it has any rescue effect on these neurons.

CHAPTER II

REVIEW OF LITERATURE

A. HYPOXIC ISCHEMIC ENCEPHALOPATHY

Hypoxic ischemic encephalopathy (HIE) refers to the clinical and neuropathological findings that occur in infants who have experienced a significant episode of perinatal asphyxia. Despite major advances in obstetric and perinatal medicine there has been little change in the incidence of HIE which has been estimated to occur in approximately 2-4 per 1,000 live, term newborn, and this incidence is even higher in small preterm neonates (Levene et al., 1985; MacDonald et al., 1980; Mulligan et al., 1980; Vannucci et al., 1990; Volpe, 1987). Depending on the gestational age and the severity of the insult, between 10-60% of asphyxiated neonates who exhibit HIE expire during the neonatal period, and of the survivors 25% or more will exhibit permanent neuropsychologic deficits like cerebral palsy, mental retardation, learning disability, and epilepsy (Ellenberg and Nelson, 1988; Freeman and Nelson, 1988; Levene et al., 1986; Low, 1988; Robertson et al., 1985, 1989).

The role of intrapartum asphyxia as the leading cause of cerebral palsy has been challenged recently in few reports (Blair et al., 1988; Freeman et al., 1988; Nelson et al., 1986). Reports from the NIH consensus conference, and American College of Obstetrics and Gynecology committee reports evaluating the antecedents

and causes of cerebral palsy (Freeman, 1985; Committee on Obstetrics, Maternal and Fetal Medicine, 1992) demonstrate that in the western industrial countries, the rate of cerebral palsy in term infants was 1 to 2 per 1000 live births in 1970, and this rate remained the same for 1990 despite modern advances in obstetrics involving electronic fetal heart rate monitoring, ultrasound, and almost complete elimination of intrapartum birth trauma by utilizing advanced technology and performing more cesarean sections (increased rate from 5% to 30%). This observation raises the possibility that a prior insult occurring earlier in gestation may have predisposed such infants to subsequent hypoxic-ischemic injury at delivery (Freeman et al., 1988). A few reports suggested that other factors such as genetic, biochemical, infectious, intrauterine environments, or other undetermined factors could be more important in the etiology of cerebral palsy (Naeye et al., 1989). Some reports indicated that no more than 10-15% of cerebral palsy cases in term infants are associated with definite evidence of severe intrapartum HI insult (Blair et al., 1988; Naeye et al., 1989). Furthermore, because newborns who sustain hypoxic injury earlier in gestation may be asymptomatic initially, the precise number of infants with cerebral injury sustained prior to delivery may be even greater. However, even if only 10-15% of patients with cerebral palsy are related directly to intrapartum HI insult, this number still constitutes a significant morbidity. It is important to emphasize that infants who sustain sufficient intrapartum HI cerebral injury to cause long-term neurologic sequelae invariably demonstrate an acute encephalopathy, which is recognizable clinically during the first week of life. Conversely, the absence of acute encephalopathy during the first week of life

essentially precludes a diagnosis of intrapartum HI cerebral injury of sufficient magnitude to cause long-term sequelae. The outcome of infants who sustain HI insult earlier during gestation and who do not display abnormal neurologic features during the neonatal period has not been established.

Perinatal Asphyxia

The primary antecedent disturbance to neuronal tissue in HIE is a deficit in oxygen supply. The perinatal brain can be deprived of oxygen by two major pathogenic mechanisms: hypoxemia, which is a diminished amount of oxygen in the blood supply; and ischemia, which is a diminished amount of blood perfusing the brain. In most instances, during the perinatal period, hypoxemia or ischemia or both occur as a result of asphyxia which refers to an impairment in the exchange of respiratory gases, oxygen, and carbon dioxide. Thus, hypercapnia, which results in a number of other metabolic (e.g., acidosis) and physiologic effects (e.g., increase in cerebral blood flow), is a major additional factor in asphyxia. Asphyxia may occur at one or more times during intrauterine and extrauterine life. In a large series of asphyxiated full-term infants studied by Brown (Brown et al., 1974), the insult was believed to have occurred primarily antepartum in 51% of cases, intrapartum in 40%, and postpartum in 9%. The clinical settings associated with asphyxia includes: (I) interruption of the umbilical circulation (cord compression or accidents); (II) altered placental gas exchange (placental abruption, previa, and insufficiency); (III) inadequate perfusion of the maternal side of the placenta (maternal hypotension, hypertension

from any cause, or abnormal uterine contractions); (IV) impaired maternal oxygenation (cardiopulmonary disease, anemia); and (V) failure of the neonate to accomplish lung inflation and successful transition from fetal to neonatal cardiopulmonary circulation.

Pathophysiology

The basic pathophysiologic mechanism of perinatal hypoxic-ischemic cerebral injury involves oxygen deprivation of cerebral tissue resulting in increased anaerobic metabolism, decreased production of energy-rich phosphate compounds, and accumulation of potentially toxic metabolites that may contribute further to the injury [e.g., excitotoxic neurotransmitter and oxygen free radicals] (Barks and Silverstein, 1992; Vannucci, 1990; Volpe, 1987). In premature human infants, the lesions are typically periventricular in location, whereas in infants born closer to term, the topography of the necrotic foci shifts to subcortical areas, often with associated selective neuronal necrosis or cavitary infarction of the convexities of the cerebral cortex as seen in the immature rat HI model (Rice et al., 1981). Possible explanation for the selective vulnerability of neurons in specific locations within the brain may relate to a combination of regional circulatory and metabolic factors, as well as the regional distribution of excitatory (glutamate) synapses.

[1] Circulatory factors. The immediate circulatory response to asphyxia in the newborn involves redistribution of systemic blood flow, with increased perfusion of more vital organs (e.g., brain, heart, and adrenal glands) and corresponding decreased perfusion of lungs, kidneys, and gastrointestinal tract. However, when the HI insult is

prolonged, this homeostatic hemodynamic mechanism fails and systemic hypotension ensues (Lou et al., 1988; Volpe, 1987). The influence of systemic hypotension on cerebral perfusion is augmented by impaired cerebrovascular autoregulation that has been observed even in the context of relatively mild/moderate perinatal HI insult (Lou et al., 1979b). Cerebrovascular autoregulation is a homeostatic mechanism which ensures the preservation of relatively constant cerebral perfusion over a wide range of systemic arterial blood pressures by means of cerebral arteriolar constriction or dilatation. The immature cerebrovascular system in the newborn infant is less capable of invoking this mechanism, presumably in part due to the deficiency of the muscular lining of cerebral arterioles. In addition, the normal blood pressure of the newborn is relatively close to the downslope of the normal autoregulation curve (Versmold et al., 1981). As a consequence, following the relatively hypoxic intrauterine environment, additional minor hypoxemia, hypercarbia, or cranial trauma associated with normal vaginal delivery may abolish this protective mechanism. Impaired cerebrovascular autoregulation results in a direct linear relationship between cerebral blood flow (CBF) and systemic blood pressure (Lou et al., 1979a, 1988). This relationship has major clinical implications because systemic hypotension will result in decreased CBF and, consequently cause ischemic cerebral injury, particularly in watershed zones of arterial supply (Lou, 1979b).

[2] Metabolic factors. It has been postulated that regional metabolic factors (e.g., localized differences in anaerobic glycolysis, energy requirements, lactate accumulation, calcium influx, or free radical formation) may play an important role in

regional vulnerability of neurons to hypoxic injury. For example, the thalamus and brain stem, which have high metabolic demands, appear to have increased susceptibility to injury (Duffy et al., 1982; Ronald et al., 1988); however, the precise relationship between regional metabolic factors and hypoxic neuronal injury has not been defined. Recent advances in magnetic resonance imaging of the newborn brain suggest that vulnerability to HI insult also may relate to active myelination in specific regions at the time of injury. Therefore, the deep gray matter structures (thalamus) and cerebral cortex that are undergoing active myelination at term are affected more severely by hypoxic insult (McArdle et al., 1987). Moreover, serial magnetic resonance imaging examinations have documented delayed myelination in regions of the brain that sustained HI injury (Johnson et al., 1987).

[3] Regional distribution of excitatory amino acid synapses. Data now indicate that excitatory amino acids, particularly glutamate, play a critical role in the mediation of hypoxic neuronal injury (Barks and Silverstein, 1992; Engelsen, 1986; Choi, 1988a; 1988b; Hagberg et al, 1987; Olney, 1978; Silverstein et al., 1987). For example, a series of tissue culture studies demonstrated that hippocampal neurons are able to survive prolonged hypoxia in the absence of glutamatergic synapses. In contrast, following the development of such synapses, all neurons died rapidly under hypoxic conditions (Rothman, 1983). In further work, synaptic blockade with glutamate blockers (e.g., magnesium, D-glutamylglycine, dextromethorphan, or ketamine) prevented hypoxic cell death (Choi, 1987, 1988b; Clark and Rothman, 1987; Rothman et al., 1987).

The critical role of glutamate is supported by experimental evidence which demonstrates that the concentration of extracellular glutamate increases dramatically within minutes following oxygen deprivation. This is a result of both excessive release from glutamatergic neurons, and impaired energy dependent glutamate uptake by presynaptic nerve terminals and glia (Benveniste et al., 1984). Two mechanisms appear to be involved in glutamate mediated cell death. In the first instance, cell death occurs within minutes as a result of activation of glutamate receptors, depolarization of the cell membrane, and intracellular influx of sodium and chloride. This is followed by passive influx of water in order to maintain osmotic balance, leading to osmotic cell lysis. Delayed cell death, which occurs within hours following the insult, may be a more important mechanism initiated by activation of glutamate receptors. This latter process involves intracellular influx of calcium through calcium ion channels, with subsequent mitochondrial dysfunction, protease activation and phospholipase activation. As a result, these enzymes degrade membrane phospholipid, release arachidonic acid, and cause excess free radical formation. The importance of the calcium-mediated mechanism of delayed cell death is supported by in vivo studies in experimental animals that indicate that neuronal death may be minimized by the administration of a glutamate antagonists or calcium-channel blockers as late as 5 minutes after cessation of the hypoxic insult (Izumiyama et al., 1988; Coacher et al., 1988). Delayed cell death, related to glutamate receptor activation, may explain the delayed ischemic neuronal death observed in both humans (Petito et al., 1987) and experimental animals (Pulsinelli et al., 1982).

Experimental studies in newborn rats have demonstrated that the cerebral concentration of glutamate increases dramatically during HI (Hagberg et al., 1987) and that systemic administration of glutamate receptor antagonists, either preceding or following hypoxic ischemic insult, appear to reduce neuronal injury and improve survival (Andine et al., 1988; McDonald et al., 1987; Thirlinger et al., 1987). In addition, the neuropathologic topography of HI neuronal injury in the mammalian brain appears to correlate with the distribution of glutamatergic synapses (Engelsen, 1986). This relationship was studied in the immature rat using a hypobaric chamber along with unilateral common carotid artery occlusion (Ikonomidou et al., 1989a, 1989b), where the similarity of cytopathological appearance and timing in both the hypobaric ischemia model and N-Methyl-D-Aspartate (NMDA) injection was demonstrated. These studies also demonstrated that the vulnerability of the immature rat brain to hypobaric ischemic damage increases during the early neonatal period (2-4 days), reaches a peak at day 6, and then diminishes progressively with increasing age. Similar findings were observed in the NMDA model.

Neuropathology

The major neuropathologic patterns of injury observed in the human newborn with HIE include (Volpe, 1987):

[1] Selective neuronal necrosis. In the term newborn this is characterized by neuronal injury of specific regions of the cerebral cortex as well as other areas such as Sommer's sector of the hippocampus, thalamus, brainstem, cerebellum, and anterior

horn cells of the spinal cord. The greater the insult, the more severe and diffuse is the neuronal injury. Circulatory factors clearly contribute to the pathogenesis of this type of injury because of the vulnerability of the vascular border zones; however, the lack of direct correlation between cerebral injury and vascular distribution suggests that other pathogenic factors may be involved. Thus, it is postulated that the rapid rate of differentiation and metabolism of neurons in brainstem nuclei and thalamus may account for the increased susceptibility of these regions to HI injury. More importantly there is increasing experimental evidence to suggest that the topography of selective neuronal necrosis corresponds closely to the anatomic distribution of glutamatergic nerve terminals.

The late neuropathologic sequelae of selective neuronal necrosis include cerebral atrophy and multicystic encephalomalacia. Affected children develop varying degrees of spastic quadriplegia, microcephaly, mental retardation, and seizures.

[2] Status marmoratus of the basal ganglia and thalamus. Injury to the basal ganglia and thalamus may be considered a subtype of selective neuronal necrosis. In this context, concomitant neuronal necrosis in the cerebral cortex has been documented in more than half of these patients. Microscopic features include neuronal necrosis, gliosis, and excessive (increased) myelination of astrocytic fibers which is responsible for the marbled appearance of the affected structures. The pathogenesis of this pattern of injury is believed to relate directly to the distribution of glutamate receptors and to the rapid differentiation of neurons in this region during the newborn period. In both newborn rats and human infants, transient, dense glutamatergic innervation of the basal

ganglia has been documented (Barks et al., 1988; Greenamyre et al., 1987). Furthermore, the administration of glutamate agonist cause neuronal injury in this region, supporting the notion that striatal neurons are particularly vulnerable to HI (Silverstein et al., 1984, 1987). Decreased uptake of glutamate has also been documented, which may relate to the high dopamine levels that are released acutely following HI insult (Silverstein et al., 1986).

The clinical features of status marmoratus of the basal ganglia and thalamus in the human newborn are not defined clearly; however, long-term sequelae include choreoathetotic cerebral palsy and intellectual impairment.

[3] Parasagittal cerebral injury. This is characterized by necrosis of the cortex and underlying white matter of the parasagittal regions of the cerebral hemispheres. The pathogenesis of this lesion is related principally to two circulatory factors: parasagittal 'water shed' zones of arterial supply of the anterior, middle, and posterior cerebral arteries and impaired autoregulation.

Clinical features of parasagittal cerebral injury observed during the neonatal period include hypotonia and weakness that is more severe proximally, especially in the upper extremities.

[4] Focal and multifocal ischemic brain necrosis. Focal brain injury occurs in approximately 15-20% of asphyxiated newborns. In addition to classic arterial occlusion, magnetic resonance imaging and cerebral angiography have recently demonstrated that cortical venous thrombosis may result in focal cerebral injury in the term newborn (Konishi et al., 1987). The pathogenesis of focal cerebral necrosis is

often multifactorial. Potential causes include proliferative vasculopathy and vascular maldevelopment of the circle of Willis, anterior choroidal, middle cerebral, and posterior communicating arteries. Arterial occlusion by embolus or thrombus has been well documented during the newborn period. Sources of emboli include placental infarction or thrombi in involuting fetal vessels, punctured or catheterized vessels. Newborn infants with cerebral infarction may be asymptomatic or may present with asymmetry of motor function or with focal or multifocal seizures.

Late sequelae include hemiplegic cerebral palsy, seizures, and intellectual impairment.

B. ANIMAL MODELS

Much of our current understanding concerning the pathophysiology of perinatal brain disorders has evolved from animal studies over the past three decades. Fetal and neonatal nonhuman primate, pregnant sheep, lamb, piglet and immature rodents have all been important animal models for perinatal brain research. Although no model can be considered 'perfect' in reflecting the variety and complexity of human brain pathology, the investigator must assess the merits and limitations of each model within the framework of the research questions being asked. From a historic perspective, the contribution of Myers and co-workers (Brann and Myers, 1975; Myers et al., 1969a, 1969b, 1972, 1975) can be considered to have laid firm foundations to the understanding of the dynamics of cerebral pathology following perinatal asphyxia. These investigators studied a variety of issues concerning asphyxial brain disorders in

the fetal and neonatal rhesus monkey after subjecting pregnant primates and the fetuses to varying types of insults at different gestational time points. Changes in the fetal cardiovascular status and the central nervous system were systematically evaluated. In addition to acute measurements, long-term behavior and neuropathologic changes in surviving animals were evaluated. The major contribution of the primate studies can be summarized as follows: [1] the immature fetal brain has a greater degree of tolerance to asphyxial insult than the mature brain; [2] in addition to the reduction in the Pa O_2 ('pure' hypoxia), ischemia is also required to cause measurable brain damage; [3] depending upon the nature and extent of HI insult, one can demonstrate a predictable array of neuropathologic changes, of which two types are quite distinct: acute nuclear damage in brain stem with global ischemia (anoxia), and edema with neuronal necrosis in the cerebral hemispheres with prolonged partial asphyxia, and [4] accumulation of lactate that occurs with glucose pretreatment is the major final common pathway for cellular damage.

Several subsequent investigators have pointed out many limitations of the primate model of Myers. These can be summarized as follows: [1] in contrast to the humans, a severe degree of maternal hypoxia is needed to cause fetal CNS damage; [2] primate experiments were done in food-deprived animals (mothers) under anesthesia and both might have significantly affected cerebral blood flow and metabolism; and [3] the most frequent neuropathologic change seen in the primate (severe brain edema followed by neuronal necrosis) is not a common feature in human perinatal HI of the term infant, while the frequently observed intra- and periventricular

hemorrhagic and ischemic lesions seen in preterm humans seldom occur in the primate. Additional limitations that have arisen in recent years concern the major restrictions placed on nonhuman primate research, particularly in the rhesus monkey. In addition in this country primate research has become prohibitively expensive, further impeding its widespread adaptation for perinatal brain research.

In 1981, Rice et al. described a modified rodent model of the Levine method (Levine, 1960). According to this modification, 7-day old postnatal rats underwent unilateral common carotid artery (CCA) occlusion under light anesthesia. After a 3- to 4- hour recovery period, the animals were subjected to 3.5 h of 8% oxygen breathing in an air-tight chamber that was partially submerged in a 37^{0}C water bath to maintain a "constant thermal environment". The pups were then removed and sacrificed at varying time periods (up to 50 h). Twenty-five animals received this treatment; over 79% of the rat pups survived, and 92% of survivors demonstrated varying degree of brain damage. Infarction of ipsilateral cerebral cortex was present in 56% of the animals, and, depending upon the duration of post-asphyxial survival, additional findings of brain damage were seen.

Confirming the work of Myers and coworkers, this study using the rat model proved that hypoxia alone did not produce histologic changes in central nervous system, but both hypoxia and ischemia were required (Rice et al., 1981). Also the clinical outcome observed at intervals up to 48 hours after hypoxic exposure differed from that observed in adult because these immature rats had no neurological, reflex or behavioral sequelae. The suggested explanation for this neuropathological damage

without corresponding clinical correlate was that motor activity in the immature rat, unlike the adult rodent, is not dependent on the cerebral cortex but rather upon subcortical structures (brain stem, cerebellum). A shift of motor control to the contralateral (intact) cerebral hemisphere (neuronal plasticity) was also suggested but seemed unlikely since neurological deficits were not observed in the immediate recovery period.

Further study of this model (Towfighi et al., 1991) was undertaken to ascertain the minimal duration of HI required to produce tissue injury and to characterize the nature and distribution of the threshold lesions which arise from HI in the immature rat. The results demonstrated that a longer duration of hypoxia was more likely to produce brain lesions and that the extent and severity of the lesion closely correlated with the length of hypoxia. Thus, no cerebral lesions were present in rats exposed to 45 minutes of hypoxia, whereas 25% (5/20) developed non-cystic lesions with 60-75 minutes exposure, and 50% (5/10) of animals with 90 minutes of hypoxia had lesions that were more extensive. White matter necrosis with relative sparing of the gray matter is a prominent feature of HI injury in experimental animals and human fetuses (Vannucci et al., 1989). This model has also been reported to produce lesions mainly in the distribution of the middle cerebral artery territory, with less involvement in the territory of the anterior cerebral artery, and none in the posterior cerebral artery territory.

The rat pup model seems to be a useful long-term survival model where the histopathologic changes occur consistently. The animal is easy to handle and available

without difficulty, and the experimental costs are modest. In addition several laboratories have utilized this model to report on several physiologic, anatomic, and pharmacological interventions characteristics in HI brain damage (Andine et al., 1988; Barks et al., 1991; Chumas et al., 1993; Hattori et al., 1990; McDonald et al., 1987, 1991; Palmer et al., 1990; Silverstein et al., 1984).

C. NEURONAL TRANSPLANTATION

Organ transplantation is an accepted treatment for renal, hepatic and cardio-respiratory diseases, and the principles of harvesting and transplantation technique, together with the maintenance of organ function, are well established. The ethical aspects of harvesting have been widely debated, and in most countries appropriate regulations have been formulated which support and indeed may encourage organ donation. As regards neural transplantation, however, these principles are yet to be agreed upon and the clinical value of such treatment is not yet established. Consequently, the management of patients with neural degenerative disease, who potentially have much to gain, is uncertain and the ethical issues are still hotly debated.

Studies during the past decade have shown that intracerebral implants of neural tissue can promote functional recovery and structural repair in the damaged CNS. Developing CNS tissue is a particularly rich source of cells for this purpose. A wide variety of glial and neuronal cell types can be obtained at different stages of development, either as multipotent precursor cells or as young differentiated neurons

with a high growth propensity. Young neurons and neuroblasts, in particular, can to some degree substitute for lost neurons in the damaged CNS, and neurotransmitter- or peptide-producing cells have successfully been used as functional systems in the brain. The neuronal transplant strategies utilized can be categorized into the following; (a) transmitter replacement, (b) hormonal replacement, (c) trophism and neuronal rescue, and (d) circuit reconstruction.

Historical Background

The first, and unsuccessful, brain transplantation was performed by Thomson in 1890 (Thompson, 1890) when he transplanted pieces of cerebral cortex from adult cats to dogs. Similar unsuccessful results were reported over the next 15 years using similar manipulation. However in 1917, Dunn (Dunn, 1917) used immature rats and demonstrated that immature neural tissue has a better chance of surviving in another brain than does adult tissue. She also noted that surviving grafts were richly supplied with new blood vessels, particularly notable with grafts placed in the ventricle of the host brain in contact with the choroid plexus, the highly vascular membrane that lines the ventricles. In the following years it became clear that effective neural transplantation was more likely to be successful using fetal donors and even more so using young rather than adult recipients (Das, 1974; Le Gross Clark, 1940). Synaptic connection between the embryonic graft and neonatal host brain was demonstrated by Lund and Hauschka in 1976 (Lund and Hauschka, 1976). Two years later, Das and Hallas (Das and Hallas, 1978) demonstrated successful transplantation of embryonic

neural tissue into adult rat brain. That was an important experiment because it implied that neural degenerative diseases which largely occur in adults could possibly be treated by embryonic neural transplants. Much of the work done in subsequent years focused on the use of neural transplantation in animal model of Parkinson's disease where dopaminergic neurons were successfully grafted into the striatum to replace degenerated dopaminergic cells within the substantia nigra. Clinical attempts to utilize these principles in patients with Parkinson's disease were reported by several investigators and, despite some setbacks, have received a wide range of publicity and enthusiasm (Backlund et al., 1985; Bjorklund et al., 1982; Hitchcock, 1989; Madrazo et al., 1988).

As far as the latter two strategies of neural transplantation (neural rescue and circuit reconstruction), many experimental reports from different laboratories have examined the survival and connectivity of fetal neocortical grafts used as a block or cell suspension, implanted in neonatal / juvenile hosts (Castro et al., 1985, 1987, 1988, 1989; Chang et al., 1984, 1986; Floeter and Jones, 1984; Lee et al., 1987; Nieto-Sampedro et al., 1982; Porter et al., 1987) or adult hosts (Gibbs and Cotman, 1987; Gonzalez and Sharp, 1987, 1988; Hohmann et al., 1988; Mufson et al., 1987; Sofroniew et al., 1986; Sorensen et al., 1990) with varying types of lesions. In the case of newborn recipients, such studies have primarily involved the homotopic transplantation of fetal presumptive sensorimotor cortex into sensorimotor aspiration cavities made immediately prior to grafting. In this work, projections from the host brain to the transplants arose from several thalamic nuclei and the ipsi- and

contralateral cortex, as well as a number of other areas including the claustrum, hypothalamus, zona incerta, basal forebrain, locus coeruleus and midline raphe (Castro et al., 1988, 1989). These projections resembled normal cortical afferents. Transplant efferents to the contralateral cortex, thalamus, striatum, pontine gray and cervical spinal cord were also found (Castro et al., 1985,1987). Other laboratories reported similar findings using similar transplant paradigms (Chang et al., 1984, 1986; Floeter and Jones, 1984, 1985; Fonseca et al., 1988; Porter et al., 1987; Santacana et al., 1990). The physiologic efficacy of graft afferents was demonstrated by studies of transplant unit activity evoked by electrical stimulation of the thalamus or forepaw (Castro et al., 1991; Neafsey et al., 1989). Pyramidal tract neurons within neocortical grafts have also been demonstrated electrophysiologically (Porter et al., 1987).

The potential therapeutic benefit of neuronal transplantation to rescue damaged neurons and reconstruct broken circuits in clinically relevant situations deserves more attention, since most studies have been devoted to the transmitter replacement strategy of transplantation in cases of the Parkinson's disease. The utilization of a more clinically relevant model that more closely resembles clinical situations would seem to be more appropriate in transplantation studies. Ischemic brain damage and infarction, a major clinical problem, both in adult and newborn human beings, is a prime candidate for this strategy. Although a few reports have addressed this possibility using adult hosts (Grabowski et al., 1992; Hadani et al., 1987; Mampalan et al., 1988; Soares et al., 1991), we are unaware of any studies as of yet that have addressed the use of neuronal transplantation in the newborn brain damaged by HI.

Studies in adult host have shown that fetal neocortical grafts survive after implantation in an infarcted area of the brain induced by MCA occlusion (Hadani et al., 1987; Grabowski et al., 1992; Mampalan et al., 1988) or concussive lateral fluid percussion (Soares et al., 1991). Evidence of graft efferent connections from the host surrounding structures were also demonstrated (Grabowski et el., 1992). The use of cortical grafts in the immature HI rat model, a clinically relevant model as discussed above, would seem to be relevant and interesting to both clinicians and scientists.

Donor-Host Relationship

The Donor-host relationship can be classified as follows:

1. Autograft, i.e., transplantation within an individual.
2. Syn- or Isograft, i.e., transplantation between genetically similar individuals of the same species.
3. Homo- or Allograft, i.e., transplantation between genetically dissimilar individual of the same species.
4. Heterograft, i.e., transplantation between different species.
5. Xenograft, i.e., transplantation between widely disparate species, e.g., across orders.

Immune Response

Peculiar to the brain, the fact that immunologic rejection of a graft in the CNS may not occur even when the donor and the recipient are genetically different, and the concept that the CNS represents an "immunologically privileged site" has evolved

since the early days of tissue transplantation to the brain (Barker et al., 1977). It is thought that because the brain lacks lymphatic vessels and lymph nodes, from which many of the cells of the immune system are deployed, and because the walls of blood vessels in the central nervous system are specialized to create a "blood-brain barrier" the access of the immune system to foreign tissue in the brain is limited. In the case of neuronal transplants the lack of rejection may also reflect the characteristics of nerve cells proper. On their surface most cells bear large molecules known as class I major histocompatibility antigens. The antigens are distinctive in each animal; they are the molecules the immune system recognizes as foreign when it rejects grafted tissue. It is known that those antigens are normally rare or absent on most neurons. The degree of immunological privilege in the brain is not absolute, and this may represent a significant impediment to the survival of histoincompatible grafts. The nature of this privilege, together with the specific immune events leading to neural grafts rejection were addressed by many investigators {see review by Sloan (Sloan et al., 1991)}. As a consequence of possible immune-mediated rejection, immunosuppression in some cases might be necessary to guarantee long-term graft survival.

Graft Survival

The survival of the neuronal graft is affected by many variables including, age and viability of donor tissue at the time of transplantation, the establishment of a blood supply, host brain condition and type of lesion used, immunological compatibility, and time of grafting in relation to the injury inflicted. Reports have shown that neuronal

grafts taken from rat fetuses at embryonic day 13-15 (E 13-15) have the greatest potential of survival after transplantation (Floeter et al., 1985; Sladek, 1984). Additionally grafts placed into neonatal hosts show more host-transplant connectivity than those found with adult hosts. However in both cases transplants have ameliorated secondary atrophy that is usually seen at distant sites from primary lesions (Huan and Cunningham, 1984, 1987; Sharp and Gonzalez, 1986; Sorensen et al., 1989). This effect was attributed to the production of trophic factors by neuronal grafts. The production of trophic factors by the host brain is also considered to be an important factor in graft survival. Relevant studies in adult rat showed that the maximum level of host trophic factors production was observed at day 10 post-traumatic brain lesion and that graft survival was maximum when grafting occurred 8 days after the lesion (Nieto-Sampedro et al., 1982, 1983). Increased transplant survival was also attributed to a decrease in the level of toxic substances released after wound lesions and to vascularization of the area by the time the delayed graft was inserted (Nieto-Sampedro et al., 1983). Studies in adult rats with brain infarction demonstrated good graft survival when implantation occurred within that window of 2-14 days after MCA occlusion (Grabowski et al., 1992; Mampalan et al., 1988) or concussive fluid percussion insult (Soares et al., 1991), but poor survival and connectivity with evidence of glial scarring at 4 weeks post insult (Soares et al., 1991). In contrast to findings using adult animals, studies on newborn rats demonstrated the survival of the transplant placed into newborn immediately after traumatic aspiration lesions of the cerebral cortex (Castro et al., 1985, 1987, 1988, 1989; Chang et al., 1984). The

determination of the optimal time for grafting seems to vary among different studies depending on the type of host and lesion used.

CHAPTER III

SPECIFIC AIMS

The present study was designed to utilize the neonatal rat model described by Rice with some refinement in order to obtain a consistent lesions. The specific aims were:

1) To assess the topography of neuronal damage induced by a modification of the rat neonatal HI model developed by Rice.

2) To determine whether or not fetal neocortical grafts will survive in the HI damaged newborn host.

3) To examine host-transplant axonal connectivity using acetylcholinesterase staining methods.

4) To examine the effect of the graft on surrounding brain structures using damage assessment scoring system and morphometric measures to see if graft has any rescue effect on neurons damaged by HI and to compare that effect if any at different time intervals after transplantation, in this case over a six week period.

CHAPTER IV

MATERIALS AND METHODS

Animals

Long-Evans, black-hooded rats were used in these experiments. Eighty animals sustained HI treatment at postnatal day 7-8 (day of birth equals postnatal day 0), and 27 of these received fetal neocortical transplants seven days after the HI insult. Survival after the HI treatment varied according to the methods used, as described in the next section, whereas all but one of the animals survived the transplantation procedure.

Hypoxic-Ischemic Insult

Using a modification of previously described methods (Rice at al., 1981), HI was induced by permanent occlusion of the right common carotid artery (CCA) combined with placement of the animals in a temperature-controlled, 8% oxygen balanced nitrogen chamber or vessel. Using methoxyflurane inhalation anesthetic, a ventral midline neck incision was made and the underlying muscles reflected to expose the right carotid sheath. The pulsating CCA was identified and isolated by blunt dissection to carefully clear surrounding tissue. The area was then bathed in saline and the artery coagulated with a microforceps cautery. The cauterized vessel was then

resected to verify complete occlusion and to prevent reperfusion. The skin wound was then sutured and the animals returned to their mothers.

Two hours after CCA occlusion surgery, individual litters (varied in numbers) were placed on an elevated plate inside an airtight glass vessel (capacity about 4.5 cubic liters) that contained a mixture of 8% oxygen balanced nitrogen. The fraction of inspired oxygen was monitored during the exposure period (range 7.6-8%) using an oxygen analyzer (Miniox ™ from Catalyst Research, Owing Mills, MD). Various hypoxia exposure times and vessel temperature were used: [I] vessel temperature was maintained at 37^0C by placing it in a temperature controlled incubator, and the animals were placed in the vessel for one hour (n=14 animals); [II] the vessel was partially submerged in a 37^0C water bath, resulting in a vessel temperature of 28-29^0C and the animals exposed to hypoxia for two hours (n=11); or [III] the vessel was partially submerged in a 45^0C water bath, resulting in a vessel temperature range of 32-33^0C and the animals placed in it for 2-2.5 hours (n=55). Temperature inside the vessel was monitored by a thermometer suspended 1-2 cm above the elevated floor that was at 37^0C . After the hypoxia treatment, animals were returned to their mothers until transplantation surgery one week later.

Neuronal Transplantation

Fetal neocortical transplants were placed in 27 animals that sustained HI insult according to the third method described above. Donor cortical tissue was obtained from embryonic day 13 (E-13) fetuses removed individually from dams anesthetized

with sodium pentobarbital (50 mg/kg). The fetuses were placed in sterile Ringer's solution at room temperature, and using microsurgical instruments, the cartilaginous fetal skull was opened and the cerebral cortex carefully stripped of meninges. Blocks of cortical tissue (1-2 mm^3) taken primarily anterior to bregma were dissected free and aspirated into a glass cannula fitted onto a Hamilton microsyringe. During this procedure the recipient animals were prepared.

Recipients were anesthetized by methoxyflurane inhalation and underwent a small 2-3 mm craniotomy on the right side at the coronal suture and 1-2 mm from the sagittal suture. With the syringe containing the donor tissue attached to a micro-manipulator, the tip of the cannula was inserted perpendicular to the surface into the host brain through the craniotomy opening, the fetal tissue was then slowly injected while withdrawing the syringe. The grafts were held in place with the bone flap previously made during the craniotomy, and the scalp incision sutured. The pups were then warmed by an incandescent lamp and returned to their mothers until weaning or sacrifice. The pregnant dams were sacrificed by intracardiac injection of sodium pentobarbital. Diagram demonstrating the transplantation procedure is shown in (Figure 1).

Histology

Animals receiving grafts were sacrificed by anesthetic overdose and cardiac perfusion with 0.1 M phosphate buffered 4% paraformaldehyde pH-7.4 at 2 (n=8), 3-4 (exactly at 24-28 days, n=11), and 6 (n=7) weeks post-transplantation. Animals that

did not receive grafts were sacrificed and perfused four weeks after sustaining HI insult. Brains were removed and immersed in the perfusion fixative overnight and then placed in 30% sucrose until they sunk. They were then stored frozen until sectioned coronally at 30 micron using a cryostat. Alternate sections were stained with toluidine blue and reacted for acetylcholinesterase (AChE) in order to identify AChE-positive axons.

AChE Histochemistry

AChE histochemistry was conducted according to a previously described technique (Ganser-Jensen, 1971; Hedreen et al., 1985). Briefly, free floating sections were first incubated in a low pH incubation mixture containing sodium citrate, copper sulfate potassium ferrocyanide, and acetothiocholine iodide. Ethopropazine (10^{-4} M, Sigma) was added to the incubation medium in order to inhibit nonspecific cholinesterase. Finally, the reaction product was intensified by brief incubations in ammonium sulfide and silver nitrate.

Brain Damage Assessments

The extent and distribution of brain damage were assessed by microscopic examination of all subserial sections. The scoring system described by Rice (Rice et al., 1981) was adapted; and the neuronal damage was graded as follows: 0, no neurons involved; 1, a few neurons involved; 2, moderate numbers of neurons involved; 3, a majority of neurons involved; and 3* = infarction. Also the degree of ventricular

dilatation at the ipsilateral compared to the contralateral hemisphere were graded as follows: 0, no ventricular dilatation; 1, mild dilatation; 2, moderate dilatation; and 3 = severe ventricular dilatation.

In addition morphometric measurements were performed at two specific coronal levels (anterior, posterior) for each animal using the computer program (NIH Image) provided by the NIH, the anterior section was taken from midway between the level of the anterior commissure and the level of the genu of the corpus callosum (= 0.70 mm from Bregma), and the posterior section from the level of the dorsal hippocampus (= -3.30 from Bregma). The two sections corresponds to plates 15, and 31 respectively of the rat stereotaxic atlas (Paxinos and Watson 1986); the anterior level included primarily the cerebral cortex and caudate-putamen; the posterior level included the cerebral cortex, hippocampus, habenula, and brain stem including the thalamus.

The following areas were measured at both the ipsi- and contralateral side of the arterial occlusion: the total sectional area of the cerebral hemisphere excluding areas of infarction if any at both the anterior and posterior sections, the sectional area of the CPu at the anterior section, the sectional area of the brain stem including the thalamus at the posterior level, and the total length of the entire CA field and the Dentate gyrus at the posterior level. Measurements were expressed in millimeters square for area measurements and millimeters for length measurements. A diagram demonstrating the two coronal levels and the structures assessed is shown in (Figure 2). The ratio of the ipsilateral (Rt) to the contralateral (Lt) brain structure measured were calculated and expressed as percent number.

Grafts Assessment

Serial examination of all animals was done, and for those receiving transplants, the size and location of the grafts was recorded. Additional comments on the connectivity of the surviving grafts were made. Graft size was assessed and scored subjectively as follow: 0, no Tp seen; 1, small or remnant poorly developed Tp; 2, moderate size well developed Tp; and 3= large size well developed Tp.

Animals were studied in two ways: First, grouped by age at sacrifice [2, 3-4, or 6 weeks after transplantation] regardless of Tp survival or size, and they were compared with control animals receiving HI only and sacrificed at 4 weeks after the insult; Second, grouped by Tp size [poor or no Tp with score of 0-1, or good Tp with score of 2-3] regardless of the age at sacrifice, and also compared with control animals receiving HI only and sacrificed at 4 weeks after the insult

Statistics

Analysis of the absolute sectional area, length measurements, and the calculated ratio between animal groups were done using Analysis of Variance for independent measures (ANOVAI) of interval scales. Analysis of the scoring system between groups was done using the Kruskal-Wallis test for ordinal scales. In addition a Correlation coefficient between the measured ratio %, and the damage assessment score was computed for all structures assessed to see if the subjective scoring method does correlate with the morphometric measurements.

Figure 1. Diagram illustrating the transplantation procedure.

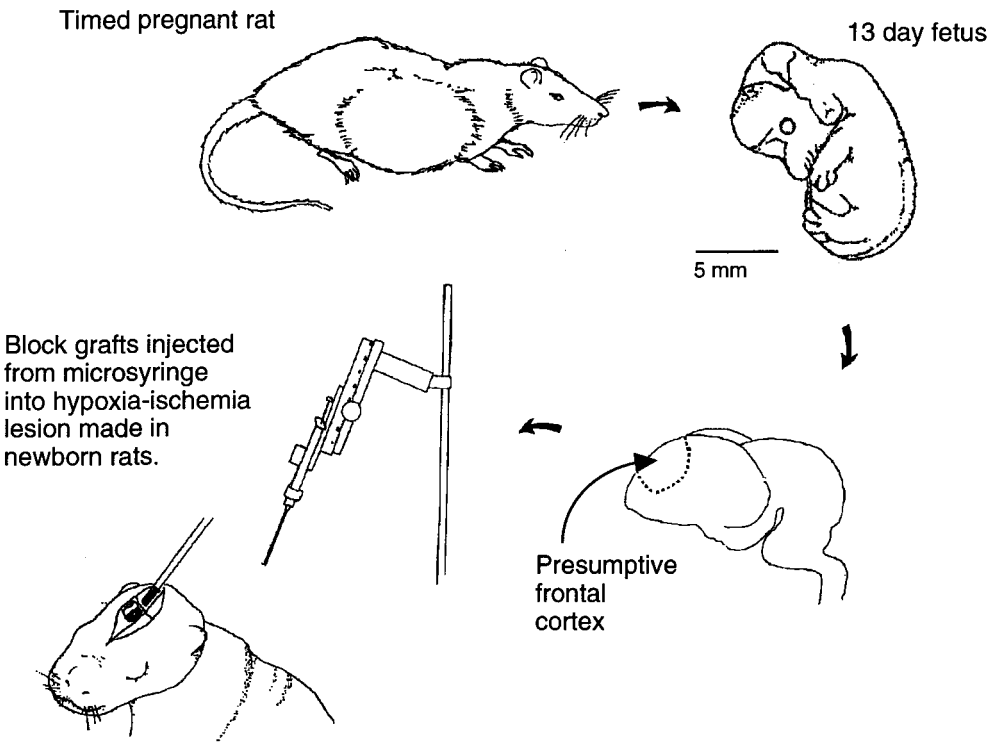
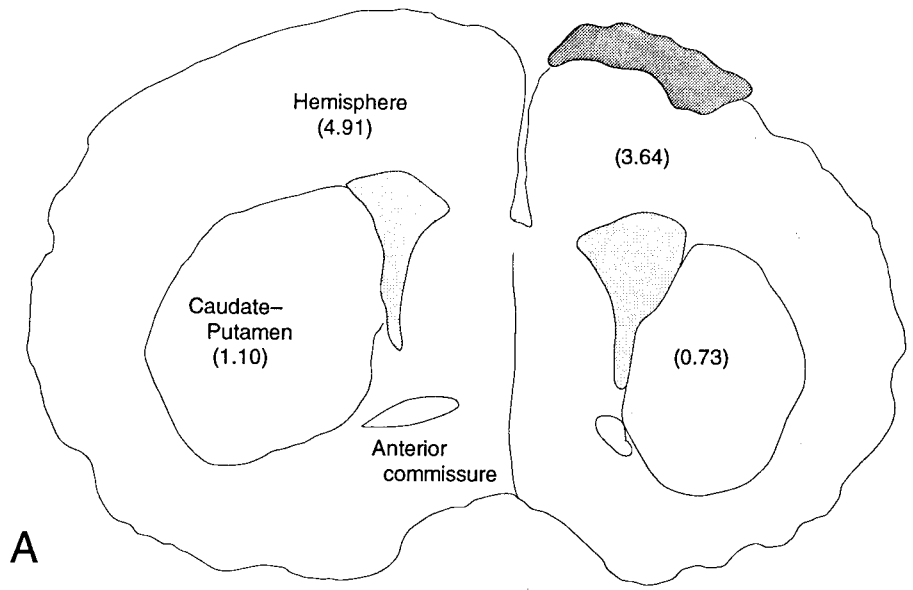
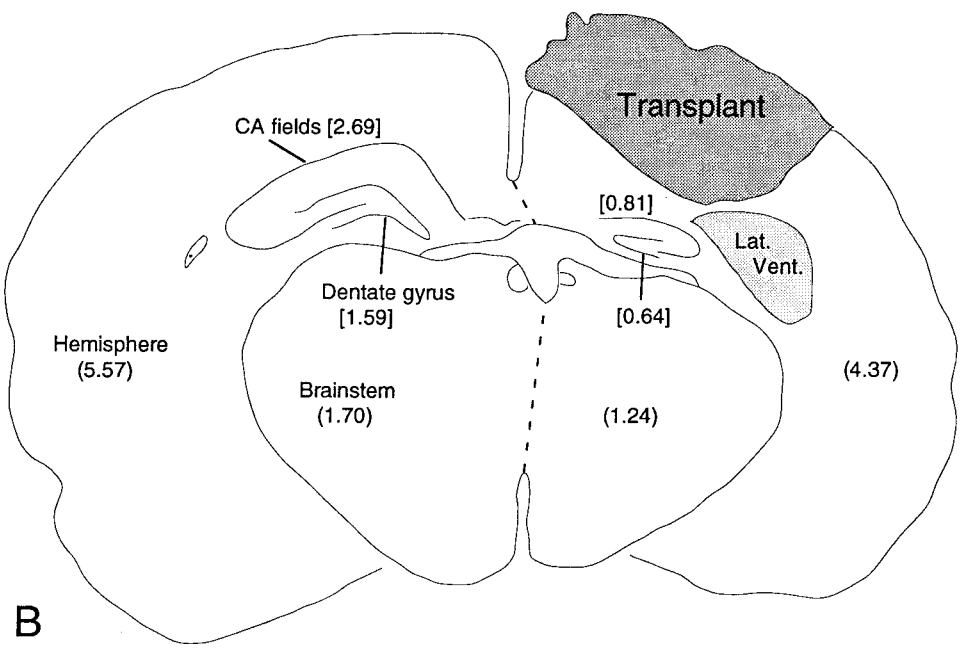

Timed pregnant rat
13 day fetus
5 mm
Block grafts injected from microsyringe into hypoxia-ischemia lesion made in newborn rats.
Presumptive frontal cortex

Figure 2. Camera lucida drawing of the two specific coronal sections where morphometric measurements were done; 2.A, anterior level showing the ipsi- and contralateral Cpu and hemispheric area measurements; 2.B, posterior level showing ipsi- and contralateral area measurements of the brain stem diencephalon and cerebral hemisphere and length measurements of the entire CA field and the dentate gyrus. area measurements in millimeter square, and length measurements in millimeter. Also note the presence of a transplant (Tp) in the ipsilateral right frontal cortex.


Hemisphere
(4.91)
(3.64)
Caudate–
Putamen
(1.10)
(0.73)
Anterior
commissure

A


Transplant
CA fields [2.69]
[0.81]
Lat.
Vent.
Dentate gyrus
[1.59]
[0.64]
Hemisphere
(5.57)
(4.37)
Brainstem
(1.70)
(1.24)

B

CHAPTER V

RESULTS

Procedures and Surgical Treatments

The overall survival results of the HI treatment groups and animals receiving Tp are presented in (Table 1). All 14 animals (2 litters with 7/litter) died during or immediately after HI treatment I, i.e., placement of the hypoxia vessel in a 37^{0}C incubator for one hour. Eight of the eleven animals (from two litters treated separately) survived using HI treatment II, i.e., partially submerging the hypoxia vessel for 2 hours in a 37-38^{0}C water bath that resulted in a vessel temperature of 28-29^{0}C. The three animals that did not survive this treatment died while in the hypoxia vessel. However, these animals all showed temporary bleeding when the CCA was cut after microcautery occlusion. Possibly, the bleeding resumed during the hypoxia period. Thirty-five of 55 animals survived HI insult according to treatment III, i.e., partially submerging the hypoxia vessel in a 45^{0}C water bath which resulted in a chamber temperature of 32-33^{0}C, and 27 of the survivors received the neocortical grafts. All but one of the grafted animals survived the transplantation surgery.

Survivors demonstrated no apparent neurological deficits immediately after the HI treatment (II, or III) or after grafting, although a moderate to severe degree of ptosis was noted in few animals at the time of sacrifice, probably due to injury of the

superior cervical ganglion during CCA occlusion. However, no detailed neurological or behavioral examination was performed.

Hypoxic-Ischemic Brain Damage

Ischemic damage, found on the side ipsilateral to the CCA occlusion, was typically mild in the eight animals surviving treatment II. In the cerebral cortex, this damage was characterized by a loss of cells, primarily in layers 3-5, in the posterior parietal and occipital areas (Fig. 3). AChE positive fibers also appeared to be less dense in the cortical ischemic areas, although this was typically more obvious in the more extensive ischemic lesions seen in animals receiving treatment III. AChE reactivity was also less dense in the hippocampus, particularly the CA1 field and the dentate gyrus (Fig. 4A, B) in treatment II animals, and corresponding Nissl staining showed the CA1 neurons to be less compacted and paler staining (Fig. 4C, D). Additionally, atrophy of the medial habenular nucleus (MHb) was obvious in several animals of this group (Fig. 4A). Damage assessment using the scoring system in animals sustaining HI according to treatment II is presented at various structures examined in (Table 2).

Table 1.-- Overall results of different hypoxic treatments and transplantation of all study animals (N=80).

Procedure	No. exposed to	No. Surviving (%)
- HI treatment I (37^0C, 1 hr)	14	0
- HI treatment II ($28\text{-}29^0$C, 2 hr)	11	8 (73%)
- HI treatment III ($32\text{-}33^0$C, 2-2.5 hr)	55	35 (63%)
-Transplantation (all had treatment III)	27	26 (96%)
Sacrificed @ 2 wk post-Tp	7	
Sacrificed @ 3-4 wk post-Tp	11	
Sacrificed @ 6 wk post-Tp	8	
- HI only (all sacrificed @ 4 wk post HI)		16
Treatment II		8
Treatment III		8

Figure 3. Low-power photomicrograph showing relatively mild cerebral cortical ischemic degeneration (arrows) from an animal that sustained occlusion of the right common carotid artery followed by 8% O2 for 2 hours at 28-29^0 C (treatment II). Nissl stain. Scale bar = 1000 u.

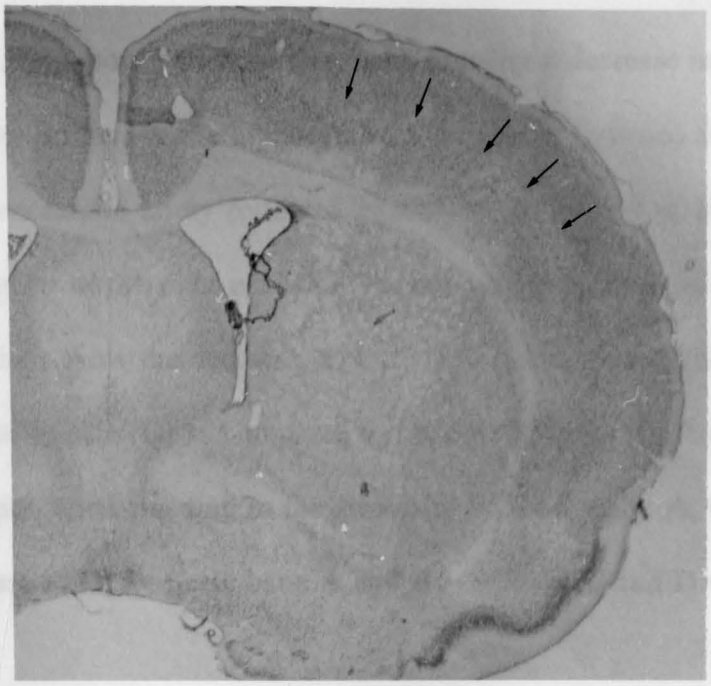
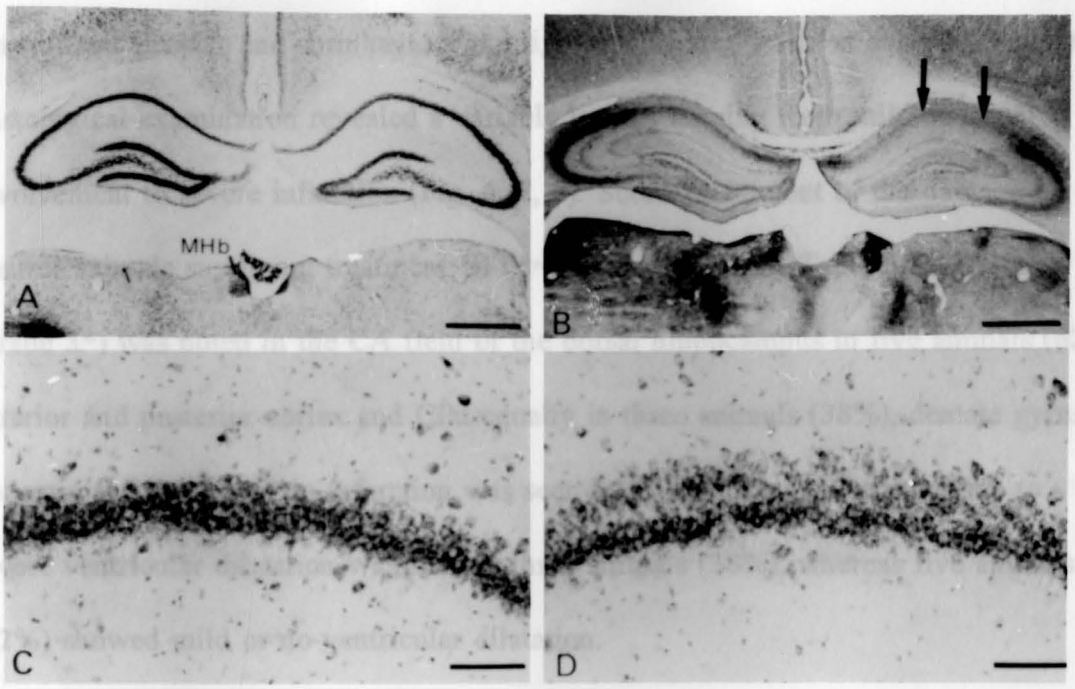


Figure 4. A and B. Low-power photomicrographs showing a decrease in acetyl cholinesterase-positive fibers in the hippocampal CA1 region (arrows) after occlusion of the right common carotid artery followed by 8% O2 for 2 hours at 28-29^{0}C (treatment II). Atrophy of the right medial habenular nucleus (MHB) is also seen in A. Higher power sections show the decrease in staining and compactness of the CA1 region on the ischemic side (D) as compared to the normal side (C). Photographs are taken from the region corresponding to the arrows in B. Nissl stain: A, C and D. Acetyl cholinesterase stain: D. Scale bars: A and B = 1000 u; C and D = 100 u.


MHb
A
B
C
D

In light of the relatively mild ischemic changes found in our initial groups of animals sustaining treatment II, subsequent animals were treated to a more severe ischemic challenge by partially submerging the hypoxia chamber in 45^{0}C water bath for 2 - 2.5 hours (treatment III). At sacrifice, gross observations commonly showed a generalized atrophy and shrinkage of the right hemisphere in these animals (Fig. 5). Histological examination revealed a variable lesions ranging from mild neuronal involvement to severe infarction (Fig. 6, 7, 8). Score assessment of the damage in control animals sustaining treatment III (n=8) is presented in (Table 3). Infarction (grade 3*) was noted in the CA field of the dorsal hippocampus in five animals (62%), anterior and posterior cortex and CPu equally in three animals (38%), dentate gyrus in one animal (13%), and no infarction was seen in the thalamus. Also moderate to severe ventricular dilatation was seen in three animals (38%), whereas five animals (62%) showed mild or no ventricular dilatation.

Figure 5. Gross view of a brain from an animal that sustained a right common carotid artery occlusion followed by 8% O2 for 2 hours at 32-33^{0}C (treatment III). Animal was sacrificed at four weeks post-transplantation. Pronounced shrinkage of the right hemisphere is seen. A fetal neocortical transplant that was placed seven days after the hypoxia-ischemia treatment can also be observed (arrows).

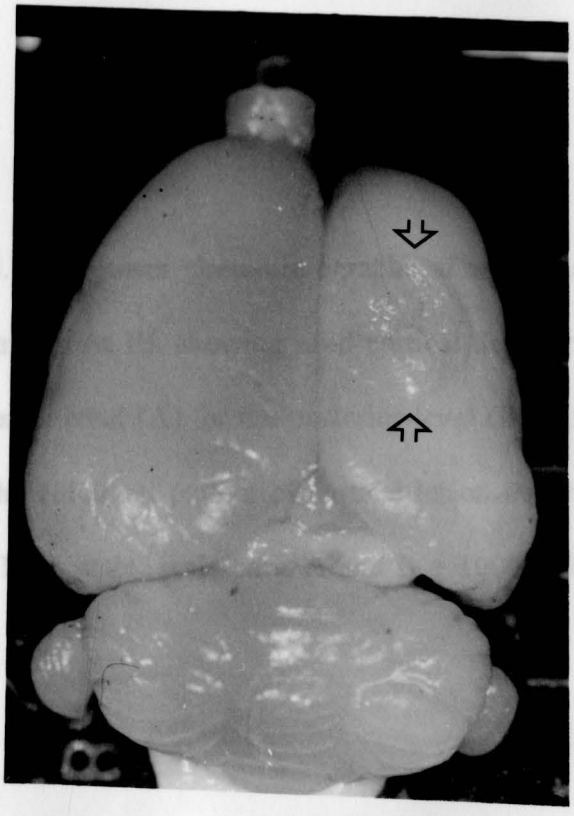
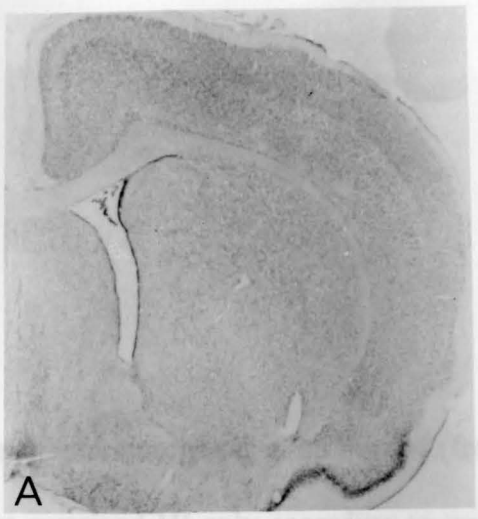
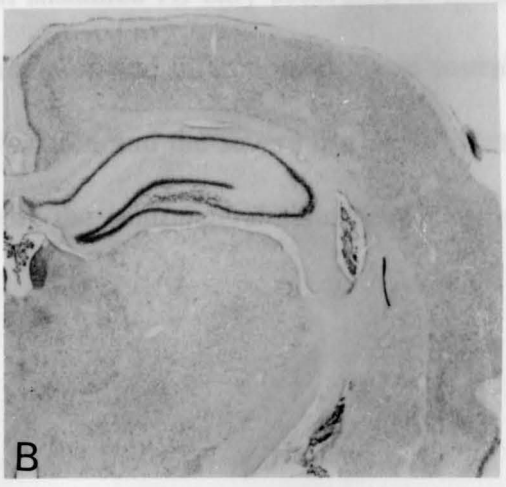


Figure 6. A-B. Low-power photomicrographs of an animal that sustained HI only according to treatment III, showing mild cortical degeneration and no infarction in either the anterior level (A), or the posterior level (B). C. high-power of B showing in milder form the columnar pattern of cortical lesion observed in animals sustaining treatment III. Nissl stain. scale bars: A and B = 1000 u; C = 500 u.


A


B

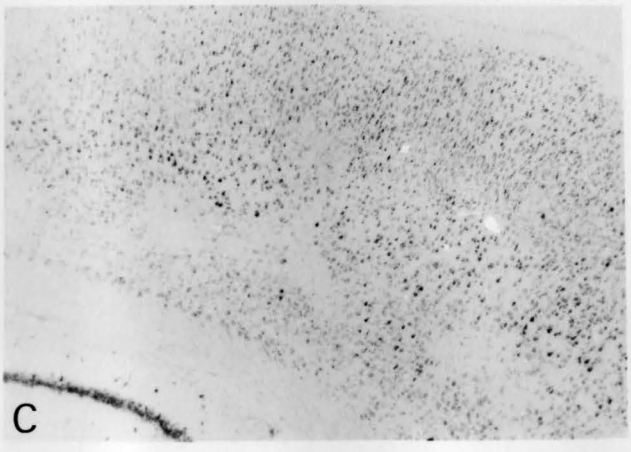
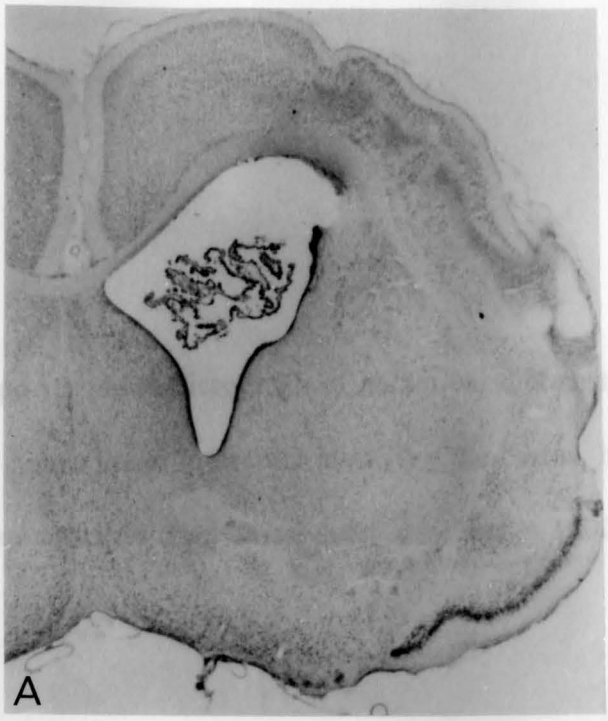

C

Figure 7. A. Low-power photomicrograph in an animal (from the same litter as animal presented in Fig. 8) that sustained HI only according to treatment III, showing moderate cortical degeneration and infarction. B. more rostral view of the same animal showing the cortical infarction and the severe ipsilateral dilatation of the lateral ventricular compared with the contralateral side. Nissl stain. scale bar = 1000 u.


A

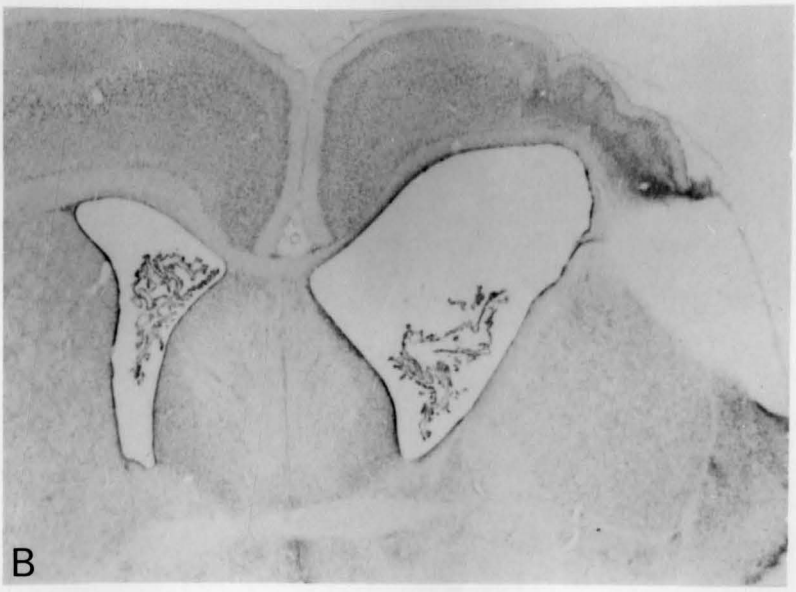

B

Figure 8. Low-power photomicrograph of an animal that sustained treatment III, showing severe hemispheric infarction involving the cortex, hippocampus, and the brain stem at the posterior level. Nissl stain. scale bar = 1000 u.

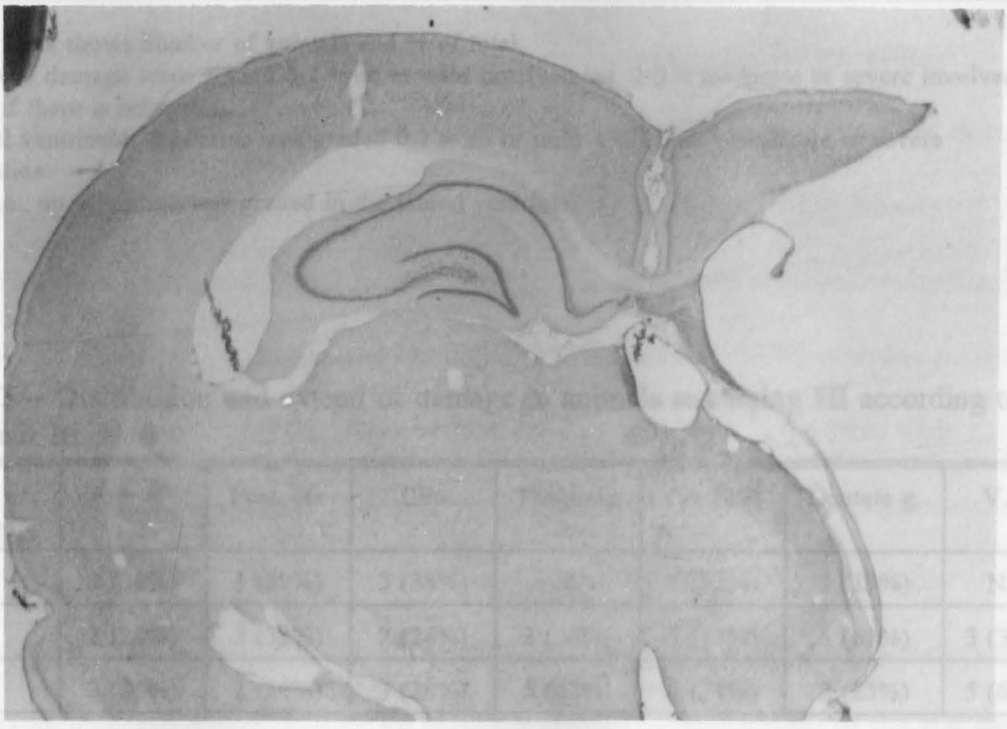


Table 2.-- Distribution and extend of damage in animals sustaining HI according to treatment II. N=8

Grade of damage	Ant. ctx	Post. ctx	CPu	Thalamus	CA field	Dentate g.	V.dil
3*	0	0	0	0	0	0	N/A
2-3	0	1 (13%)	1 (13%)	0	0	0	0
0-1	8 (100%)	7 (87%)	7 (87%)	8 (100%)	8 (100%)	8 (100%)	8 (100%)

Note: Data shows number of animals and % of total.
- Neuronal damage were graded 0-1 = no or mild involvement, 2-3 = moderate or severe involvement, and 3* if there is infarction.
- Lateral ventricular dilatation was graded 0-1 = no or mild V.dil, 2-3 = moderate or severe V.dilatation.
- N/A i.e., no infarction was graded in the lateral ventricle.

Table 3.-- Distribution and extend of damage in animals sustaining HI according to treatment III. N=8

Grade of damage	Ant. ctx	Post. ctx	CPu	Thalamus	CA field	Dentate g.	V.dil
3*	3 (38%)	3 (38%)	3 (38%)	0	5 (62%)	1 (13%)	N/A
2-3	2 (24%)	3 (38%)	2 (24%)	3 (38%)	1 (13%)	5 (62%)	3 (38%)
0-1	3 (38%)	2 (24%)	3 (38%)	5 (62%)	2 (24%)	2 (25%)	5 (62%)

Note: Same data report and grading system as in table 2.

The more typical ischemic damage seen microscopically in animals receiving treatment III is presented in (Fig. 9A, B) . The extensive cerebral cortical atrophy is indicated by marked decrease in cortical thickness seen on the side ipsilateral to the CCA occlusion. This atrophy involved the frontal, parietal, temporal and occipital areas of the dorsolateral convexity, showing a progressive rostra-caudal increase in severity (Figs 10 A-G) and confirming the primary involvement of the MCA territory in this model. Neuronal loss, which typically extended across lamine 2-6, often showed a columnar patterns, with spared neurons found in association with blood vessels (Fig. 9A). The columnar sparing of neurons was often more apparent in rostral cortical areas where ischemia was generally less severe. An increased density of AChE-positive fibers was found in association with the spared neuronal columns (Fig. 9B), and this is clearly distinguished from the more uniform AChE staining patterns found in the host cortex of the opposite hemisphere (Fig. 9C). Animals with extensive ischemic neocortical lesions also demonstrated severe dorsal hippocampal atrophy affecting all CA fields and the dentate gyrus (Fig. 10 C-F) with infarction in 62% and 13% of animals respectively. Corresponding to the hippocampal neuronal loss, AChE-positive fibers showed an increase density of projections to the remaining neurons, particularly in the dorsal blade of the dentate gyrus (figs. 11C, D and 11). The ventral hippocampus appeared to be unaffected except for distortions caused by the overall hemispheric shrinkage (Fig. 10G).

Pronounced atrophy of the caudate-putamen was also evident (Fig. 10A). Additionally, neuronal loss was especially marked in several thalamic nuclei including

anterodorsal (AD), anteroventral (AV), reticular (RT), parataenial (PT), ventrolateral (VL), ventral posterolateral (VPL) and ventral posteromedial (VPM) nuclei (Figs 9 and 10). prominent degeneration showing an obvious glial reaction was found in the dorsolateral geniculate (DLG) and the lateral posterior (LP) thalamic nuclei (figs 9 F and 10 C,D).

Figure 9. A. Photomicrograph from ischemic cortex demonstrating the typically observed columnar sparing of neurons (arrows) that are found near blood vessels seen in longitudinal section in this figure. B. Histological section adjacent to A shows patches of cholinesterase-positive axons (arrows) seen in proximity to blood vessels (asterisks). The commonly observed patchy distribution of cholinesterase-positive axons in ischemic cortex differs from patterns observed in the opposite normal cortex as seen in C. Nissl stain: A; Acetyl cholinesterase stain: B and C. Scale bars: A and C = 500 u; B = 200 u.

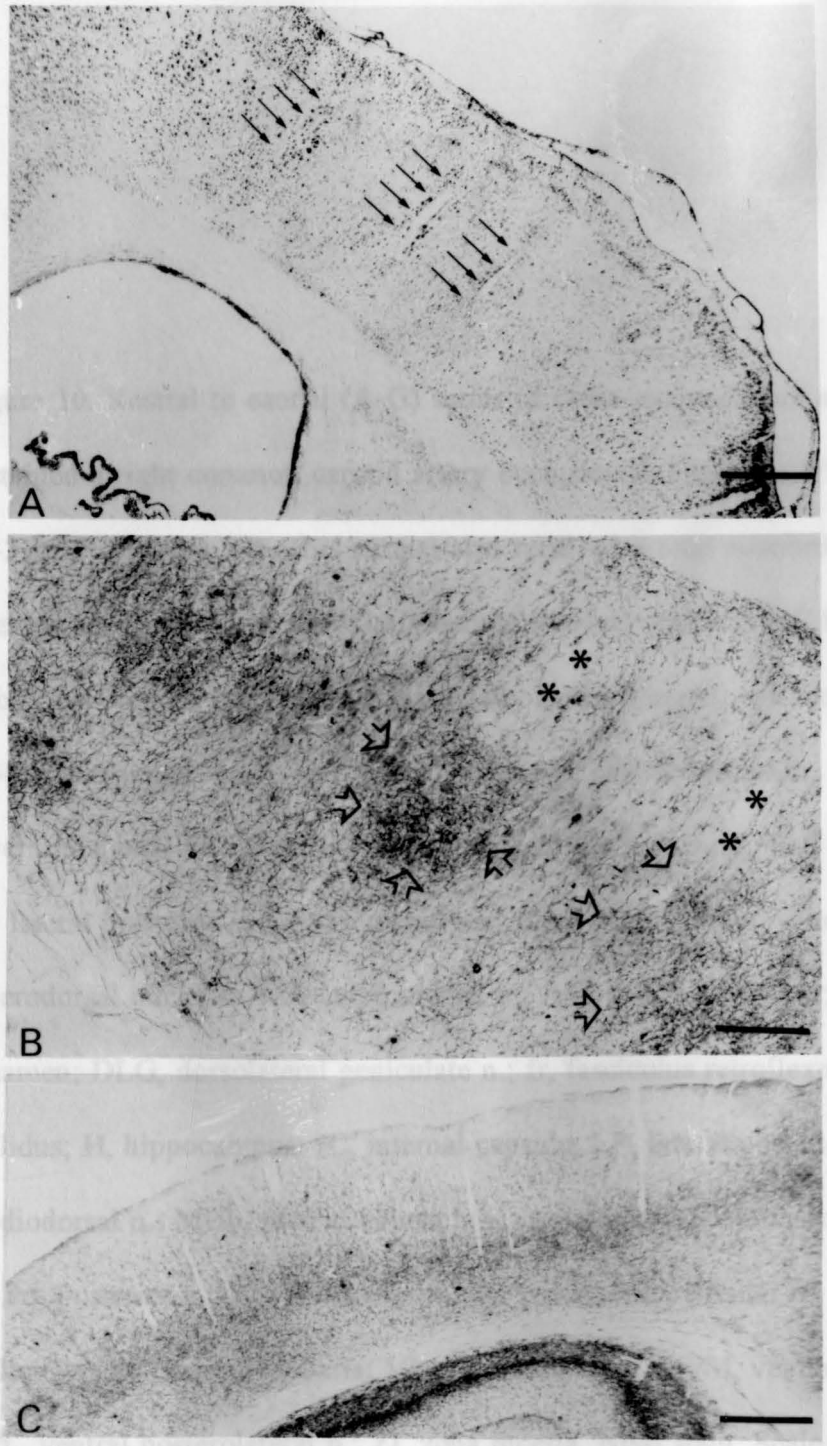

A
B
C

Figure 10. Rostral to caudal (A-G) series of cross-sections from an animal that sustained a right common carotid artery occlusion followed by 8% O2 for 2 hours at 32-33^{0}C (treatment III). This animal also received a fetal neocortical transplant (dashed lines) at one week after hypoxia-ischemia. Marked neuronal cell loss and cortical atrophy is seen on the right side especially in the parietal and occipital cortical areas (D-G). Prominent atrophy of the hippocampus, caudate-putamen, medial habenula and several thalamic nuclei is also seen. Apparent, dark staining glial reactivity is seen in the lateral posterior and dorsolateral geniculate nuclei (E,F). Abbreviations: AD, anterodorsal nucleus; AM, anteromedial n.; AV, antero ventral n.; CP, caudate-putamen; DLG, dorsolateral geniculate n.; fr, fasciculus retroflexus; GP, globus pallidus; H, hippocampus; IC, internal capsule; LP, lateral posterior n.; MD, mediodorsal n.; MHb, medial habenula n.; pc, posterior commissure; PF, parafascicular n.; Po, posterior n.; PT, parataenial n.; R, red n., RT, reticular n.; SC, superior colliculus; sm, stria medullaris; VL, ventrolateral n.; VPM, ventral posteromedial n.; VPL, ventral posterolateral n.; ZI, zona incerta. Nissl stain. Scale bars = 1500 u.

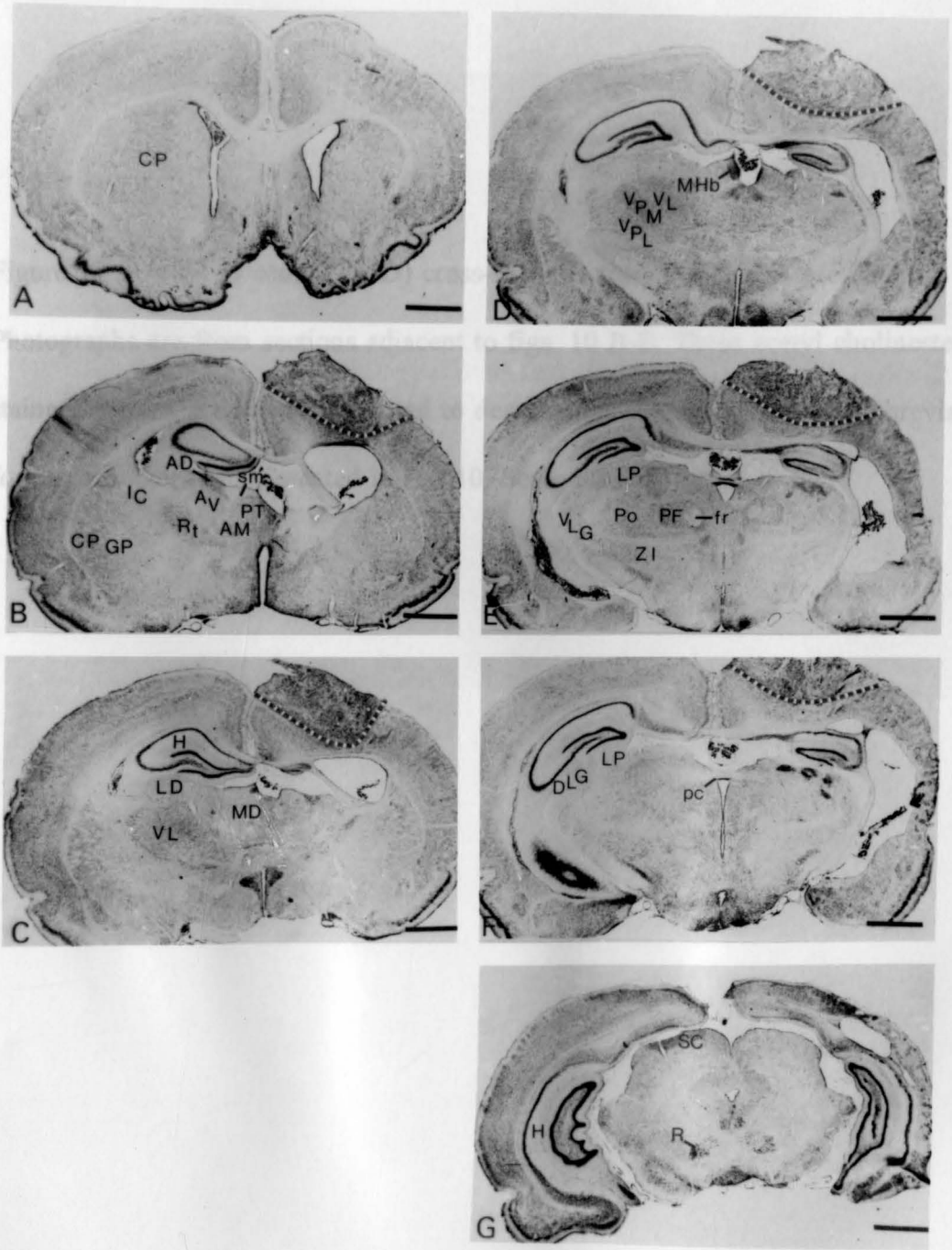

CP
A
MHb
VP
VL
M
VPL
D
AD
sm
IC
AV
PT
Rt
AM
CP GP
B
LP
VLG
Po
PF
fr
ZI
E
H
LD
MD
VL
C
DLG
LP
pc
F
SC
H
R
G

Figure 11. Rostral to caudal (A-D) cross-sections from the animal presented in Fig. 10. Photographs are from sections adjacent to figs. 10 B-E. These acetyl cholinesterase stained sections are especially suited to demonstrate thalamic atrophy. Abbreviations: f, fornix; all others are presented in fig. 10. Scale bars = 1000 u.

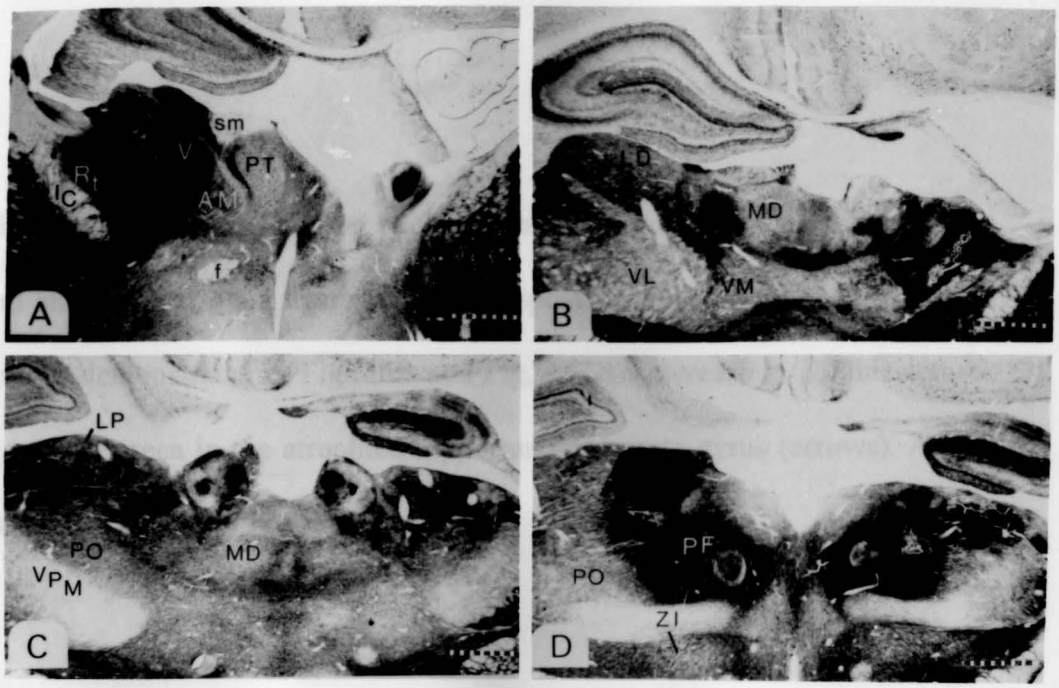
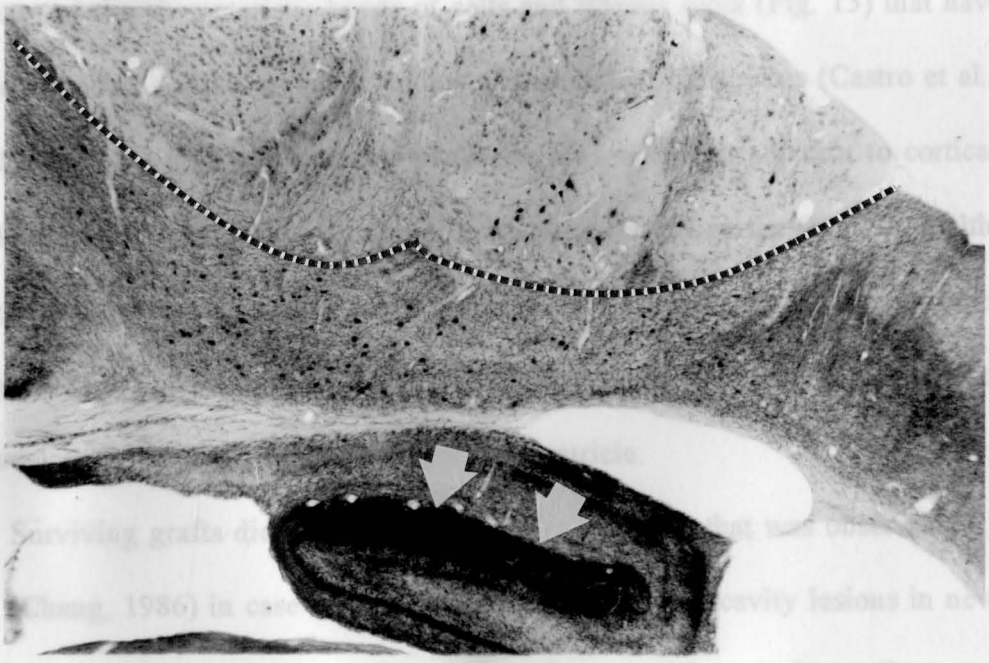

sm
V
PT
Rt
IC
AM
f
A
D
MD
VL
VM
B
LP
PO
MD
VPM
C
PF
PO
ZI
D

Figure 12. Numerous, dark-staining cholinesterase positive neurons seen within the transplant and the host brain near the host-transplant interface (dashed) line. These cells are also demonstrated in Fig. 15B and Fig. 16. An increase in cholinesterase positive axons is seen in the atrophied hippocampal dentate gyrus (arrows). Acetyl cholinesterase stain. Scale bar = 500 u.



Grafts Survival and Morphology

All but one of the 27 animals receiving grafts survived the transplantation procedure, and transplants were identified histologically as well developed (size 2-3) in 16 animals (62%), or poorly developed (size 1) in 5 animals (19%) . The remaining 5 animals showed no evidence of a graft. With Nissl stain, clearly identified transplants demonstrated the characteristic bands of cells and fibrous septa (Fig. 13) that have been described in several previous studies of neocortical transplants (Castro et al., 1985, 1988, 1989). The grafts were typically located within or adjacent to cortical areas demonstrating ischemic damage of the parieto-frontal cortex (Fig 10B), although three grafts were located superficial to the host cortex (2 well developed grafts in the midsagittal area, and one poorly developed graft in the right frontal cortex) and one well developed graft extended into the lateral ventricle.

Surviving grafts did not show the laminated pattern that was observed by Chang (Chang, 1986) in case of cortical transplantation into cavity lesions in newborn rats. They appeared as a collection of condensed neurons that can be easily differentiated from surrounding host tissue. However, crossing axons were seen in the periphery of the grafts. Grafts were found in animals demonstrating moderate ischemic lesions (Fig. 5, 10) as well as in animals that showed extensive infarcts (Fig. 14A).

AChE-positive fibers were observed within most grafts including those placed in animals with extensive infarct lesions (Fig. 14B). These fibers were sparse or absent in the small superficial grafts (Fig. 15A, B), but they showed an extensive distribution in grafts located within the host cortex (Fig. 16) or the lateral ventricle (Fig. 15C).

Their presence in such grafts and the absence of AChE fibers in superficial grafts indicates that these fibers were derived from the host CNS and were not intrinsic to the transplants. A marked increase in AChE-positive neuronal soma was found consistently in the host tissue adjacent to the transplants (Figs. 12, 15B and 16). AChE reactive cells were also often found within the transplants, particularly near the transplant-host interface (Fig. 12).

Figure 13. Photomicrograph of a transplant showing the characteristic whorls of cells and white matter septae (asterisks). Nissl stain. scale bar = 200 u.

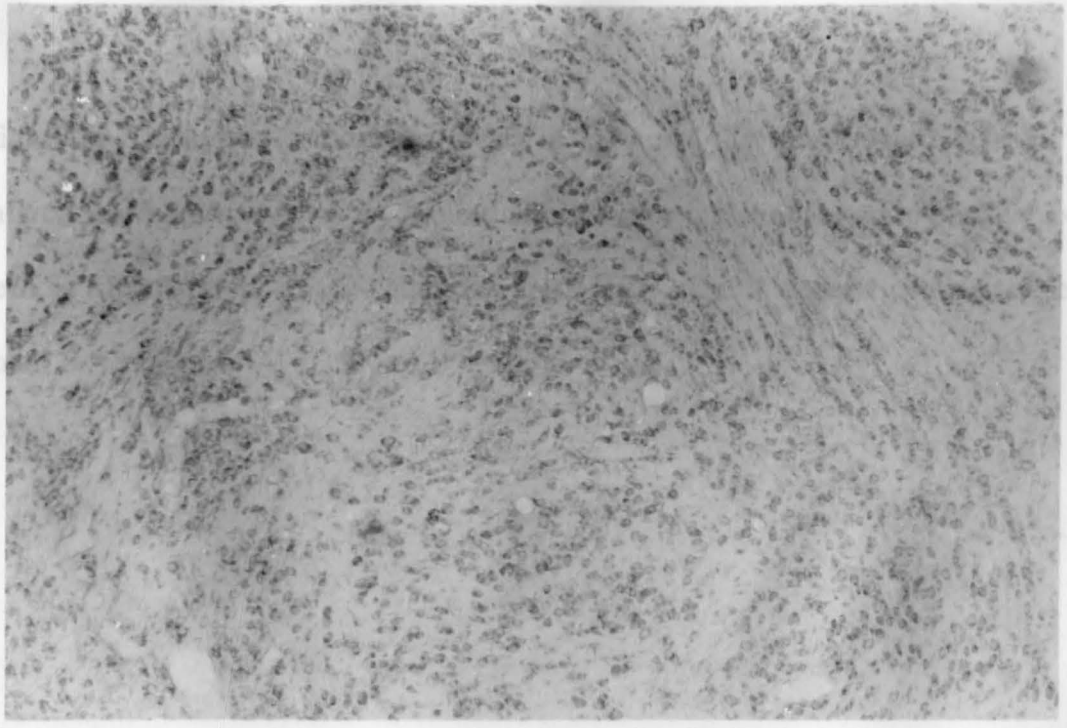


Figure 14. Transplant (asterisk) surviving in an animal that demonstrated a large infarct. B. Higher power of the same transplant showing cholinesterase positive axons, seen as irregular dark fibers, within the graft. Nissl stain: A; Acetyl cholinesterase stain: B. Scale bars: A = 2500 u; B = 200 u.

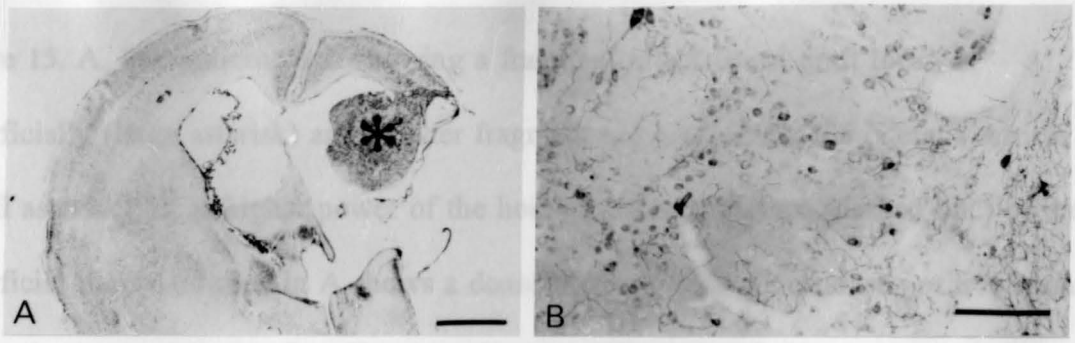

A
B

Figure 15. A. Photomicrograph showing a fragment of a cortical graft located superficially (large asterisk) and another fragment growing within the lateral ventricle (small asterisk). B. A higher power of the host-transplant interface (dashed line) of the superficial transplant seen in A shows a dense network of cholinesterase positive axons within the host cortex but few fibers are found within the graft. Numerous dark-staining cholinesterase positive neurons are seen in the host cortex near the transplant. C. In contrast to the superficially located transplant, dense cholinesterase fibers are seen within the graft located in the lateral ventricle. LV, lateral ventricle. Nissl stain: A; acetyl cholinesterase stain: B and C. Scale bars: A = 1000 u; B = 200 u; C = 300 u.

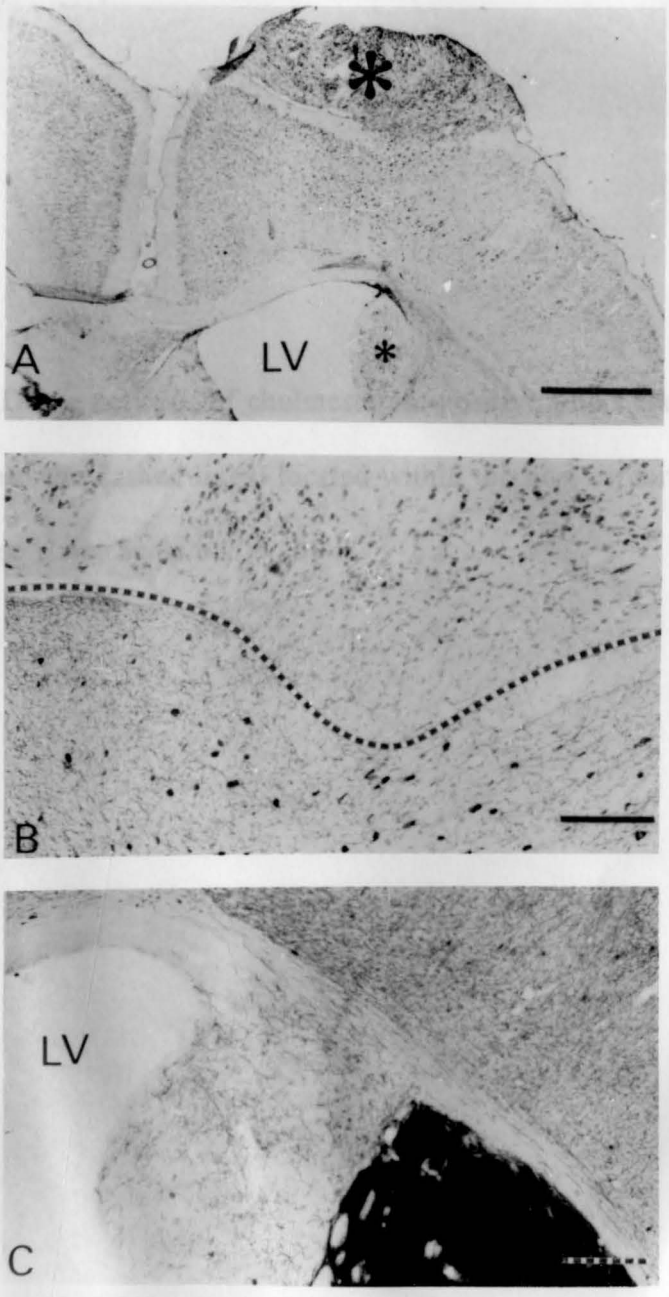
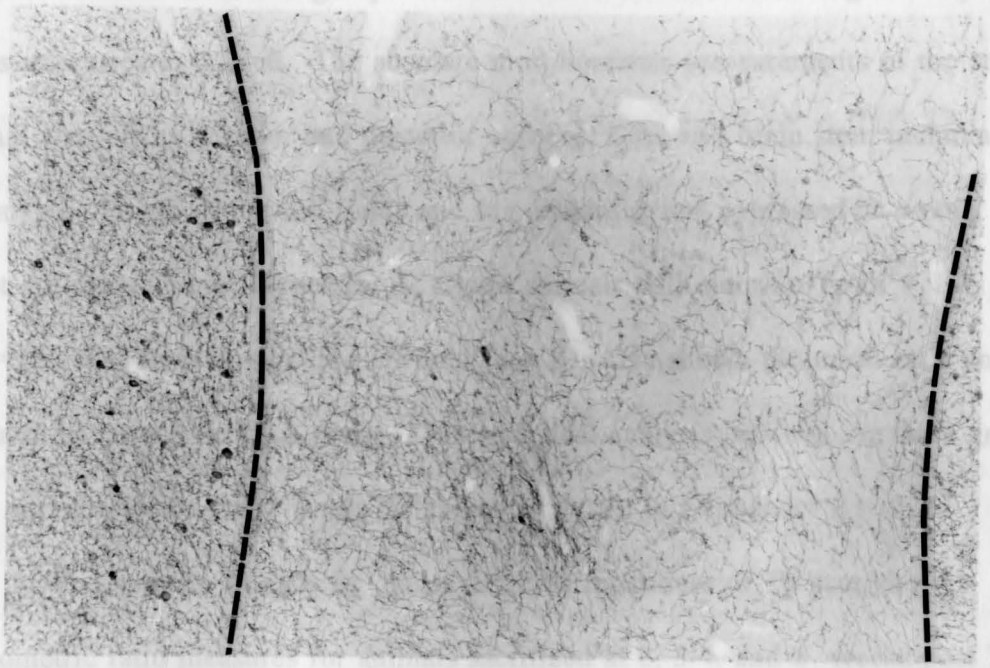

A
LV
*
B
LV
C

Figure 16. Dense network of cholinesterase-positive fibers found within a neuronal graft (between the dashed lines) located within the host cerebral cortex. Acetyl cholinesterase stain. Scale bar = 200 u.



Brain Damage in Transplanted Animals

The damage assessment scores of animals that underwent transplantation are presented for animals with good Tp (Table 4) and for animals with poor/no Tp (Table 5). As shown the pattern of damage in terms of its distribution or extent is similar in the two groups and the statistical analysis (see Appendix B) revealed no significant difference between these two groups and the control animals sustaining HI only in various structures examined . The absolute morphometric measurements of the cross sectional area of the anterior and posterior cortices, CPu, and brain stem including the thalamus, the length of the CA fields and the dentate gyrus, expressed as a ratio percent are presented in (Appendix A, C) for animals with poor/no Tp (n = 10), good Tp (n =16), and control receiving HI only (n = 8). Also shown the mean ratio and (Standard deviation) for each group. The statistical analysis between the three groups which also revealed no significant difference is shown in (Appendix B).

In studying the animals by age at sacrifice regardless of Tp size, the morphometric ratio measures for animals sacrificed at 2, 3-4. and 6 weeks post Tp, and comparing them to the control animals sacrificed at 4 weeks post HI insult are presented in (Appendix A, C). Using the damage assessment scoring system the extend of brain damage in animals receiving Tp seem to shift from moderate to severe (grade 2-3) at 2 weeks to no or mild (grade 0-1) at 6 weeks post transplantation (Figures 17-19) compared to animals sustaining HI only (Figure 20). However, statistical analysis revealed no significant difference between the three groups and control animals using either the ratio measures or the scoring system (Appendix B).

Table 4.-- Distribution and extent of damage in animals with good Tp (size 2-3). N=16

Grade of damage	Ant. ctx	Post. ctx	CPu	Thalamus	CA field	Dentate g.	V. dil
3*	2 (13%)	7 (44%)	2 (13%)	1 (6%)	8 (50%)	5 (31%)	N/A
2-3	9 (56%)	5 (31%)	10 (62%)	6 (38%)	8 (50%)	10 (62%)	4 (25%)
0-1	5 (31%)	4 (25%)	4 (25%)	9 (56%)	0	1 (6%)	12 (75%)

Note: Data shows number of animals and % of total.
- Neuronal damage were graded 0-1 = no or mild involvement, 2-3 = moderate or severe involvement, and 3* if there is infarction.
- Lateral ventricular dilatation was graded 0-1 = no or mild V.dil, 2-3 = moderate or severe V.dilatation.
- N/A i.e., no infarction was graded in the lateral ventricle.

Table 5.-- Distribution and extent of damage in animals with poor/no Tp (size 0-1). N=10

Grade of damage	Ant. ctx	Post. ctx	CPu	Thalamus	CA field	Dentate g.	V. dil
3*	2 (20%)	3 (30%)	2 (20%)	1 (10%)	3 (30%)	1 (10%)	N/A
2-3	4 (40%)	5 (50%)	7 (70%)	2 (20%)	7 (70%)	6 (60%)	4 (40%)
0-1	4 (40%)	2 (20%)	1 (10%)	7 (70%)	0	3 (30%)	6 (60%)

Note: Same data report and grading system as in table 6

Figures 17, 18, 19, 20. Bar graphs showing the distribution and extend of neuronal degeneration using the semi-quantitative scoring system, in transplanted animals regardless of transplant size and sacrificed at 2, 3-4, 6 weeks after transplantation, figs 17, 18, 19 respectively, and for control animals with treatment III HI only fig. 20. Neuronal degeneration were graded as described before; 0 or 1= no or mild neuronal involvement, 2 or 3= moderate or severe degeneration, and 3* if there is infarction.

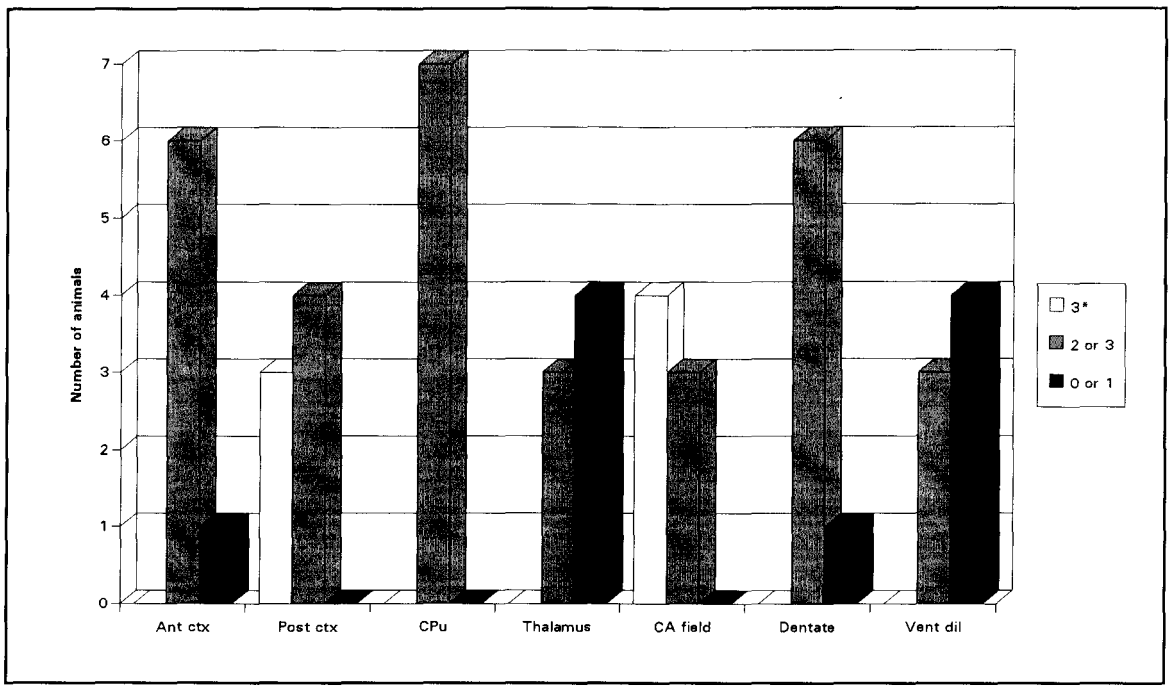


Figure 17. Distribution and extend of neuronal degeneration 2 wks post-transplantation. (N=7)

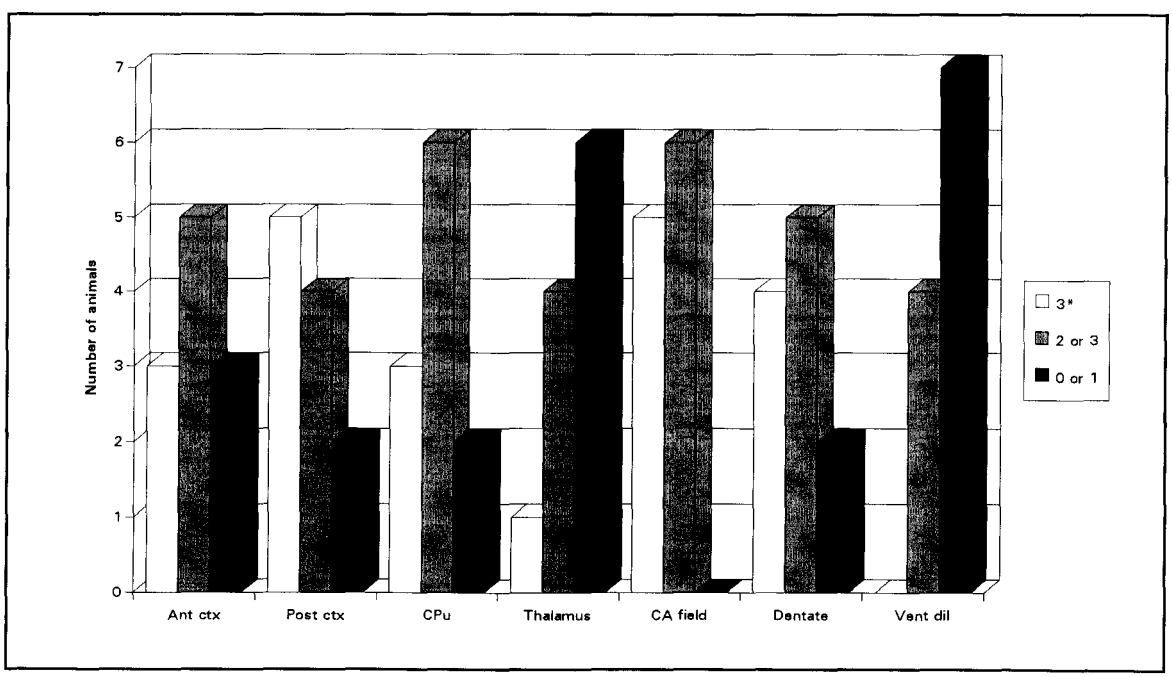


Figure 18. Distribution and extend of neuronal degeneration 3-4 wks post-transplantation. (N=11)

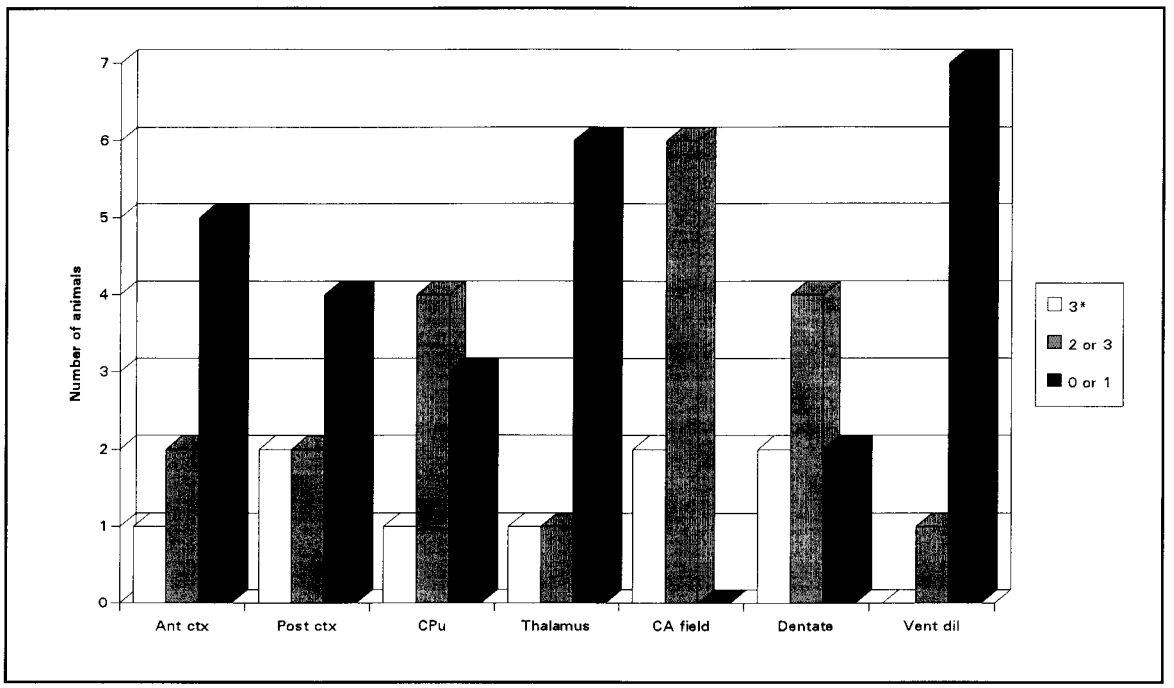


Figure 19. Distribution and extend of neuronal degeneration 6 wks post-transplantation. (N=8)

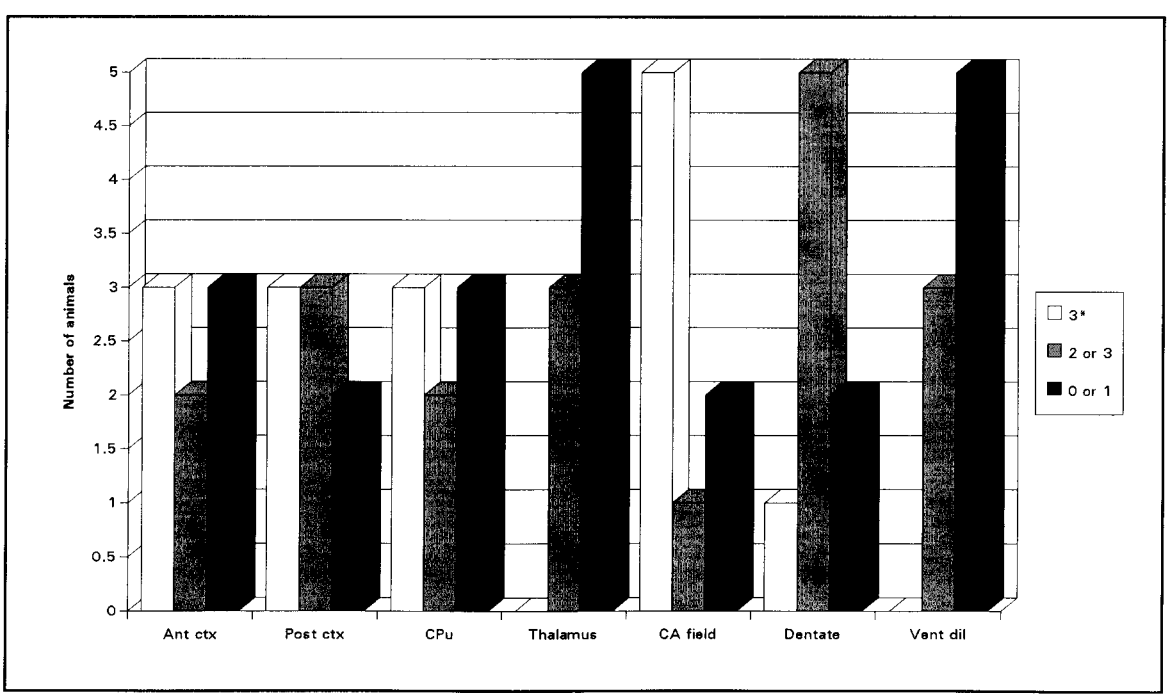


Figure 20. Distribution and extend of neuronal degeneration in control animals with treatment III HI only, no transplant attempted. (N=8)

The above data show that, there were no difference in the distribution and the degree of brain damage in animals without and with transplant regardless of the size or how developed the Tp is. In addition, post transplant survival period had no significant influence on the degree or distribution of brain injury.

A plot of all assessed scores and the corresponding ratio measures for the six structures examined in all surviving animals (n = 34) [excluding animals with HI according to treatment II (n=8) as they all had mild damage] was done in order to compare the subjective semiquantitative scoring system with the morphometric measurements (Figure 21). As shown the mean ratio of any score was significantly different from the other means (ANOVAI, $P < 0.0001$). In addition a good overall negative correlation was noted between the score and the ratio ($r = -0.78$, $P < 0.0001$), i.e., the lower the score the higher the ratio (indicating minimal or no damage), and the higher the score the lower the ratio (indicating severe damage). See Appendix C for details.

Figure 21. Plots of the subjective semiquantitative scores for various structures assessed (N=6), and the corresponding ratio measures for all animals surviving HI according to treatment III with and without transplantation (N=34), i.e., there are 204 data points representing percent ratios (6 x 34 = 204). Arrows indicate the mean ratio of each subjective score and the standard deviation (SD).

** Using ANOVAI and follow up with fisher exact t-test, there was a significant difference between the mean (arrows) of all scores. In addition a negative correlation between the scores and the ratio was found, r=-0.78.

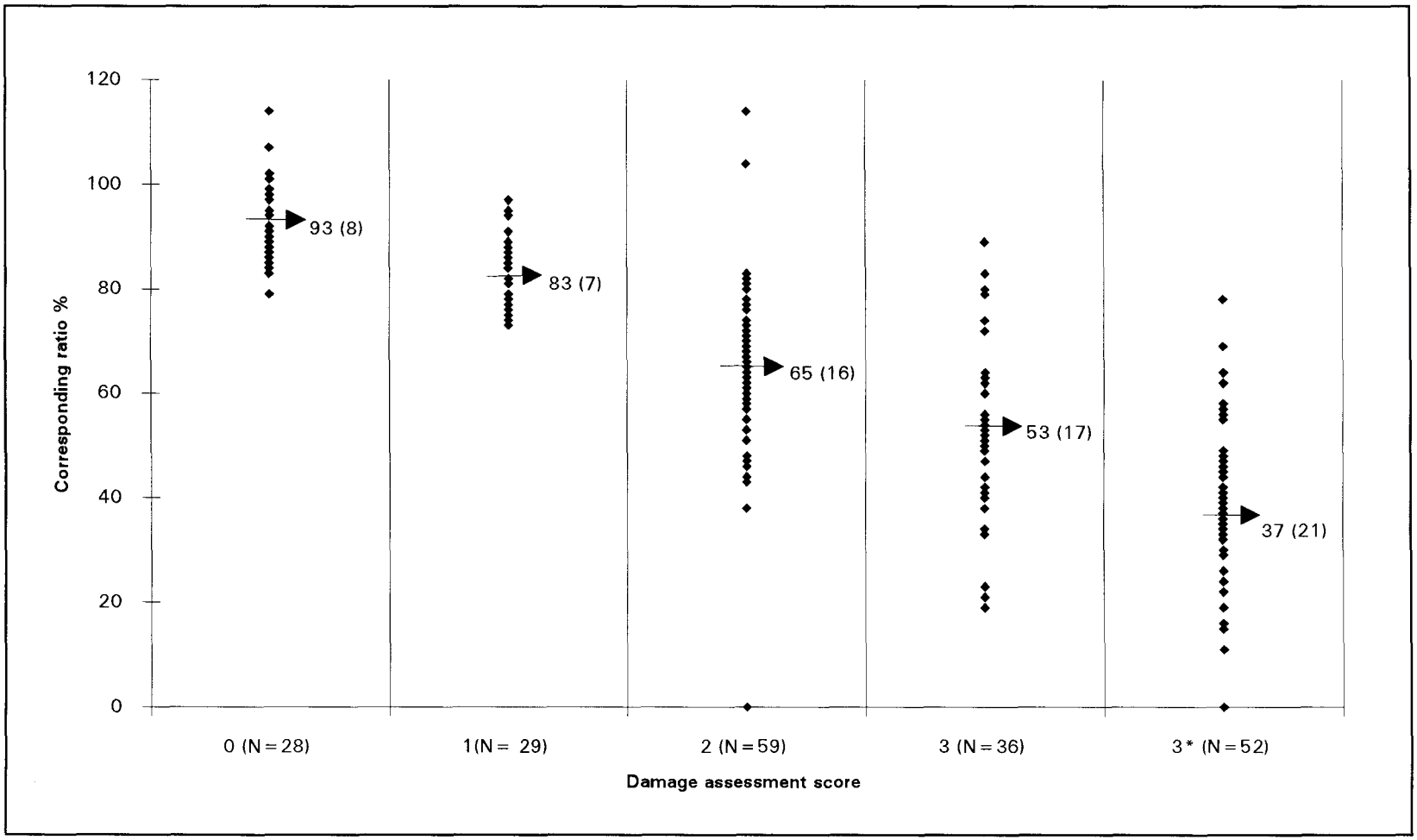


Figure 21. Plots of subjective scores and corresponding ratios for all animals surviving treatment III HI, arrows indicate mean (SD).

CHAPTER VI

DISCUSSION

Hypoxic-Ischemic Injury

Variations of the perinatal hypoxia-ischemia model described by Rice (Rice et al., 1981), have been used in several recent experiments (Andine et al., 1988; Barks et al., 1991; Burke et al., 1991; Chumas et al., 1993; Ford et al., 1989; Hattori et al., 1990; McDonald et al., 1987, 1991; Mujsce et al., 1990a, 1990b; Palmer et al., 1990; Towfighi et al., 1991). These studies invariably involved unilateral occlusion of the common carotid artery in 6-10 day old rats (although reference to the day of birth as being 0 or 1 is often not indicated) followed typically by a 2-4 hr recovery period before exposure to an hypoxic environment for 2-3 hrs. The hypoxia is usually achieved by placing the animals in a closed vessel that contains 8% oxygen and which is partially submerged in a 37^{0}C water bath. In our initial attempts at duplicating these procedures, we found that exposing seven day old rats to 8% oxygen for 1 hr with a temperature in the hypoxic vessel maintained at 37^{0}C caused a 100% mortality in two litters (n=14). We subsequently found that partial submersion of the hypoxia vessel in a 37^{0}C water bath, resulting in a 28-29^{0}C vessel temperature, led to mild damage and survival rates of 73%. In related work involving unilateral common carotid artery occlusion in neonatal rats followed by exposure to a hypobaric chamber partially

submerged in a water bath maintained at 38^{0}C, the mean axillary temperature of the rats was 34.5^{0}C (Ikonomidou et al., 1989a, 1989b). Based on our observations, this temperature is presumed to lie intermediate to the hypoxic chamber and the water bath temperatures. The mild brain damage observed in HI insult according to treatment II (Table 2) in which animals were exposed to a lower temperature than treatment III (28-29^{0}C versus 32-33^{0}C), confirms the report of the protective effect of hypothermia in animals sustaining HI insult (Dietrich, 1992; McDonald et al., 1991).

In order to obtain a more significant neuronal damage, we exposed newborn rats to a more severe insult by increasing water bath temperature to 45^{0}C, which resulted in a vessel temperature of 32-33^{0}C for 2-2.5 hrs. This treatment (III), which is a modification of the original model, resulted in a more severe identifiable brain damage in most animals. The survival rate of (63%) until sacrificed (up to 8 weeks after insult), compared with the varying degree of brain damage in 92% of animals and survival rate of 79% (all sacrificed at 48 hours after insult), in the original model.

The observed pattern of brain damage obtained by this modification was closely similar to previous work (Rice et al., 1981). In addition, we noted atrophy of the medial habenular nucleus and severe dorsal hippocampal involvement affecting all CA fields including the CA2 which was consistently preserved in the original model. dorsal hippocampal with infarction was noted in 62% of animals, while the ventral hippocampus showed no or mild involvement. The dentate gyrus was involved in over 75% of our animals with infarction in 13%. No infarction of the thalamus was seen, and we also noted ventricular dilatation of moderate to severe magnitude in 38% of

animals. The difference in survival rate and the distribution of damage between our animals and the original model may be attributed to the higher temperature of our hypoxia chamber, genetic susceptibility as we have used Long-Evans, black-hooded rats versus Sprague-Dawley used originally.

The subsequent brain damage observed in animals sustaining treatment III showed considerable variation even between litter mate pups (Figures 6, 7). This is in agreement with reports in human newborn (Volpe, 1987) as well as experimental animals (Duverger, 1988). Factors which may contribute to this variation include: individual susceptibility, variation in the core temperature of individual pups, arterial blood pressure and intracranial perfusion pressure, and glucose homeostasis. Individual core temperature was not measured in our litters but was assumed to be within a narrow range in each litter placed in the hypoxia vessel. However, temperature variation between rats at different areas of the chamber because of their proximity or farness to the water bath, could predispose animals with higher core temperature to a more sever damage than those with lower core temperature (Dietrich, 1992). Arterial blood pressure and intracranial perfusion pressure, which is the product of mean arterial systemic blood pressure minus the intracranial pressure, can aggravate the induced damage by producing further ischemia in case of low perfusion pressure, or hemorrhage in case of high perfusion pressure (Vannucci et al., 1990; Volpe, 1987). Glucose homeostasis before, during, or after the HI insult has been shown to have a detrimental effect on the degree and extent of brain damage (Duffy et al., 1982; Hattori et al., 1990; Vannucci et al., 1989). In our experiment rat blood glucose was

not measured or monitored, but this could vary among individual pups. In addition another variable concerns the volume of the hypoxia chamber. In the present experiment, litters of varying numbers (8-14) pups were placed in hypoxia vessels with 4500 ml capacity, resulting in a variable volume for every pup; this differs slightly from the original model where 2- 3 pups were placed in a 500 ml chamber. All these detailed factors discussed above were not addressed or monitored in the original model. In addition individual variability was also noted by Rice (Rice et al., 1981). The base line variability in the degree of brain damage among individual rats; may have masked any effect of grafts on rescuing surrounding brain structures. Previous work demonstrated a transplant mediated amelioration of thalamic atrophy induced by well defined aspiration lesions made in newborn rats (Sharp et al., 1986; Sorensen et al., 1989).

Survival and Morphology of Cortical Grafts

Well developed grafts were observed in 16 animals sustaining hypoxia according to treatment III, giving a survival rate of 62%, and, if poorly developed transplants were counted, the observed survival would be over 80%. This corresponds to the transplant survival rate (75%) reported using block grafts in adult rats with ischemic brain damage after MCA occlusion (Mampalan et al., 1988) and newborn rats with cavity lesions (Sorensen et al., 1992) 75% and 73% respectively, but less than the 100% survival reported using cell suspension grafts in adult rats with ischemic brain damage (Grabowski et al., 1992). Obviously multiple injection of cell suspension graft

had a better survival than the single injection of block grafts.

Our observed transplant survival rate in the hypoxic-ischemic environment with its associated correlates of brain edema, lymphocytic infiltration, delayed neuronal loss, and decreased CBF with subsequent decrease of blood supply to the grafts, that corresponded to other reports of transplantation into different newborn or adult hosts, indicates that HI does not seem to have a major negative influence on graft survival. This was clearly demonstrated when we observed well developed Tp surviving and exchanging axons in hosts with severe brain damage and infarction of almost the whole ipsilateral hemisphere (Figure 14A). These results are contrary to findings in adult rats with MCA occlusion (Mampalan et al., 1988) where poor transplant survival was noted in animals with large infarction.

The survival of grafts implanted at seven days after the HI insult concurs with previous reports using adult rats; the seven day post ischemia interval falls within the described optimal post-lesion window of 2-14 days (Grabowski et al., 1992; Mampalan et al., 1988; Nieto-Sampedro et al., 1983; Soares et al., 1991). In preliminary work not included in this report, grafts placed at two days after HI appears to show less survival while grafting at 14 days showed almost similar survival rates to grafting at seven days after injury. Possible explanation for reduced graft survival with short post HI intervals could be attributed to an increased level of neurotoxic substances released at the site of injury which are maximum in the first five days after injury (Nieto-Sampedro et al., 1983). Also, neurotrophic factors may have been insufficient at two days in that such factors were shown to reach maximal levels at the site of lesion six

days after the injury in adult rats (Nieto-Sampedro et al., 1982). Additionally, longer survival may be necessary for collateral circulation to develop and become effective in providing adequate blood supply to the graft.

Fetal donor tissues used in the present study were obtained from the presumptive sensorimotor cortex and implanted into the fronto-parietal cortex of the host, i.e., homotopic grafts, and such grafts have been shown previously to make connections with the host brain and become electrophysiologically functional (Castro et al., 1985, 1987, 1988, 1989; Neafsey et al., 1989). Presumably, the surviving grafts observed in our HI recipients developed similar connection, but this was not examined.

The morphologic appearance of the grafts corresponded to previous reports (Castro et al., 1987, 1988, 1989; Sorensen et al., 1989) in showing characteristic whorls and bands of cells separated by fibrous septae, which were commonly quite prominent, and stood in clear distinction from the surrounding host tissue. Although a normal cortical lamination in both homotopic and heterotopic cortical grafts obtained from E-15 donor and placed into newborn rat cortex with aspiration lesion has been reported in one study (Chang et al., 1986), the absence of normal lamination in our grafts concurs with multiple reports involving the placement of grafts in newborn rats with aspiration lesion (Castro et al., 1987, 1988, 1989, 1991) or adult rats with brain damage induced by MCA occlusion (Grabowski et al., 1992; Mampalan et al., 1988).

The size of the transplant did not vary according to the post transplantation survival period, or the degree of HI damage, nor did size appear to correlate with transplant location or proximity to structures such as the ventricular ependyma or pial

membrane. These findings suggest that other factors such as trophic effect, blood supply, host age, or other undetermined factors play more important roles in newborn host. In addition, the observation that graft survival or size did not vary over the six week period from the time of transplantation indicates that graft survival is determined in the early post transplantation period (as early as 2 weeks).

AChE Histochemistry

AChE positive fibers were seen consistently crossing the graft-host interface in the majority of animals with good or with poor Tp which provided evidence of transplant-host connectivity. The presence of AChE-positive fibers in most grafts located within the host cortex or lateral ventricle and their absence in superficial grafts (Figure 15A, B) indicated that these fibers were derived from the host CNS and were not intrinsic to the transplant. In addition, the decreased density of AChE fibers on the side of grafts adjacent to the area of HI cortical damage (Figure 16) suggests that the damaged cortex was less able to promote the growth of axons into the transplants. The extrinsic source of AChE fibers observed in our animals are in agreement with previous studies demonstrating that cholinergic afferents grow into homotopic neocortical grafts placed into cortical aspiration cavities or excitotoxic lesions in both neonatal and adults animals (Castro et al., 1988, 1989; Hohmann et al., 1988; Sorensen et al., 1990). Although the origin of these fibers was not established in our study, previous work suggested that they originate from the host basal forebrain (Castro et al., 1988).

The presence of cholinesterase reactive cells within the transplants corresponds to findings involving the placement of fetal cortical transplants into adult rats with brain infarction (Mampalan et al., 1988). However, contrary to this previous report, we consistently observed even higher concentration of cholinesterase positive cells within the host brain near the host-transplant interface. As discussed previously (Mampalan et al., 1988), the presence of reactive cholinesterase cells within the graft could reflect altered expression of the AChE enzyme in transplanted neurons, which could occur in several ways: 1) the transient increase in expression of AChE that occurs during normal development of thalamic and cortical pathways may persist in transplants; 2) transplanted neurons may synthesize more AChE enzyme in the presence of host trophic factors, and 3) transplantation could induce the AChE enzyme in normally AChE-negative neurons. The cause of increased concentration of reactive cells in the host brain near the host-transplant interface in our experiment is unclear, but could reflect a positive trophic effect exerted by the graft on this newborn host and a transient phase where host brain is in the process of extending axonal connections to the graft.

Neuroprotective Effects

A graft mediated amelioration of the atrophic thalamic nuclei has been reported after cortical transplantation into newborn animals that sustained traumatic aspiration lesions (Sharp et al., 1986; Sorensen et al., 1989). Although our findings showed an apparent trend toward less severe damage with increased post transplantation survival,

no statistically significant neuroprotective effects exerted by the grafts on host brain structures was found. Possibly, the variability of brain damage observed among individual rats precluded the possibility of observing graft mediated rescue of host brain areas damaged by HI. Further study of the possible neuroprotective effect of transplants in HI animals requires the development of a more consistent lesion model.

CHAPTER VII

CONCLUSIONS AND SUMMARY

Conclusions

1. Unilateral common carotid artery occlusion followed by exposure to 8% oxygen at postnatal day seven causes variable brain damage similar to reports concerning human newborns that sustain hypoxic-ischemic insult.
2. A good negative correlation was found between the subjective semi-quantitative damage assessment scoring system and the more objective absolute ratio measures in the areas demonstrating neuronal damage. These two scoring systems used separate or in combination are useful and accurate tool in assessing and quantitating brain damage.
3. Fetal neocortical block grafts survive transplantation into neonatal rats that sustained hypoxic-ischemic insult. The survival and overall graft development was not dependent on the degree of ischemia and subsequent brain damage in recipient host.
4. Grafts are able to form connections with host brain as evidenced by acetylcholinesterase AChE positive fibers observed crossing the host-graft interface. While the descriptive details of these connections were not studied in this experiment, the origin of these fibers appears to be similar to previous

transplant studies showing such inputs to arise from within the host CNS.

5. There was no significant correlation between the survival, size, and integration of transplant noted with post-transplantation survival period. However, transplant survival seems to be more favorable when grafted at 7 days in comparison to 2 days after HI insult.

6. The potential neuroprotective effects of transplants on neurons threatened by hypoxic-ischemic insult was not observed using semi-quantitative assessment or morphometric measures. We speculate that this is due to the wide range of variability in damage produced by HI among individual animals.

Summary

The present findings demonstrate that fetal neocortical grafts can survive in the HI treated newborn rat. However, the clinical application of this technique at the present time remains a rather remote treatment strategy for brain damage after perinatal HI, but further animal studies may uncover information leading to clinical applications not involving neuronal grafting. For example, since thalamic rescue has been demonstrated following the immediate grafting into cortical aspiration lesions in newborn rats, a similar response may also be observed using the HI lesion model. The ability of delayed grafts to reduce secondary neuronal degeneration would demonstrate that such neurons are responsive to delayed treatment. While related studies, albeit using adult animals, demonstrated that cell loss occurred rapidly after cortical aspiration lesions, thalamic cell death was more protracted beginning 14 days after

excitotoxic-induced lesions (Ross et al., 1990). This is likely related to the sparing of unmyelinated axons within the area of excitotoxic injury (Erselius et al., 1991). In light of considerable evidence indicating that excitotoxic accumulation plays a pivotal role in HI induced cell death (Barks et al., 1991), we speculate that axonal sparing will prolong thalamic cell survival as compared to axotomizing aspiration lesion. Accordingly, cortical grafts placed one week after HI insult may still be within the temporal window where thalamic neurons may form connections with the transplants and thus be rescued. This determination is considered especially important to future work directed toward the development of therapeutic strategies to reduce secondary neuronal degeneration.

APPENDIX A

Table 6.--Detailed morphometric ratio in various structures assessed of individual animals in the control group with HI only (treatment III). N=8

Animal	Ant.ctx	Post.ctx	CPu	B.stem	CA field	Dentate g	V. dil
936A	89%	90%	84%	86%	87%	81%	0
936B	73%	80%	51%	84%	58%	50%	0
936C	84%	84%	77%	95%	61%	53%	0
937A	56%	24%	48%	40%	0%	0%	2
935B	71%	77%	68%	74%	44%	71%	0
815G	55%	42%	46%	64%	36%	33%	2
815H	95%	104%	98%	101%	102%	107%	0
815I	69%	49%	57%	70%	64%	63%	3
Mean (SD)	74.0 (15)	68.8 (27)	66.1 (19)	76.8 (20)	56.5 (31)	57.3 (32)	

Note: - Ratio shown is of ipsilateral /contralateral side (Rt /Lt) expressed as a percent. Also shown the mean (SD) of ratios for each structure assessed.
- Ventricular dilatation was assessed on the ipsilateral side relative to the contralateral one and scored as follows; 0 = no dilatation, 1 = mild, 2 = moderate, and 3 for severe dilatation.

Table 7.--Detailed morphometric ratio in various structures assessed of individual animals in the group with poor/no transplant. N=10

Animal	Ant ctx	Post ctx	C - Pu	B stem	CA field	Dent gyrus	V dil	Tp size	Tp location
797A	62%	32%	61%	60%	34%	44%	2	1	Rfc sprf
797B	71%	74%	58%	91%	35%	53%	2	1	Rfc
797G	78%	82%	67%	94%	82%	97%	0	0	NA
797H	73%	81%	51%	84%	34%	53%	1	1	Rfc
811E	57%	74%	0%	94%	72%	59%	1	0	NA
811F	79%	76%	65%	87%	38%	43%	0	1	Rfc
811G	95%	92%	88%	99%	72%	91%	0	1	Rfc
936F	78%	91%	58%	84%	83%	73%	0	0	NA
935A	57%	33%	58%	41%	23%	21%	2	0	NA
937B	35%	26%	22%	55%	11%	0%	3	0	NA
Mean (SD)	68.5 (17)	66.1 (26)	52.8 (25)	78.9 (20)	48.4 (26)	53.4 (30)			

Note: - Ratio measures, mean (SD), and ventricular dilatation were assessed as described in Table 8.
- Transplant (Tp) size was assessed as follows; no Tp seen = 0, poor developed small size = 1, well developed moderate size = 2, and well developed large Tp is 3.
- Transplant location was assessed as described in the Table.

Table 8.--Detailed morphometric ratio in various structures assessed of individual animals in the group with good transplant. N=16

Animal	Ant ctx	Post ctx	C - Pu	B stem	CA field	Dent gyrus	V dil	Tp size	Tp location
797C	51%	38%	46%	58%	16%	41%	1	2	mid saggital
797D	63%	80%	47%	75%	42%	63%	1	3	mid saggital
797E	76%	80%	76%	82%	42%	55%	0	3	Rfc
797F	54%	47%	44%	61%	47%	54%	3	2	Rfc
797I	60%	63%	38%	69%	47%	60%	0	3	Rfc
797J	114%	114%	99%	89%	62%	69%	3	3	lateral V
797K	74%	78%	66%	73%	30%	40%	1	3	Rfc
811A	60%	65%	48%	76%	52%	70%	0	2	Rfc
811B	62%	66%	57%	83%	0%	0%	1	3	Rfc
811C	39%	19%	15%	33%	0%	0%	3	3	Rfc
811D	78%	87%	70%	85%	60%	55%	0	3	Rfc
811H	90%	97%	79%	98%	64%	73%	0	3	Rfc
811I	91%	90%	97%	88%	89%	79%	0	2	Rfc
811J	56%	64%	40%	67%	19%	22%	1	3	Rfc anterior
936D	57%	37%	45%	49%	72%	48%	2	3	Rfc
936E	86%	85%	83%	81%	40%	29%	0	2	Rfc
Mean (SD)	69.4 (20)	69.6 (25)	59.4 (23)	72.9 (17)	42.6 (25)	47.4 (24)			

Note: -Measures, mean (SD), and ventricular dilatation were assessed as described in Table 8.
- Transplant (Tp) size and location were assessed as described in Table 9.

Table 9.--Detailed morphometric ratio in various structures assessed of individual animals in the group sacrificed 2 weeks post-transplantation. N=7

Animal	Ant ctx	Post ctx	C - Pu	B stem	CA field	Dent gyrus	V dil	Tp size	Tp location
797A	62%	32%	61%	60%	34%	44%	2	1	Rfc sprf
797B	71%	74%	58%	91%	35%	53%	2	1	Rfc
797C	51%	38%	46%	58%	16%	41%	1	2	mid saggital
797D	76%	80%	47%	75%	42%	63%	1	3	mid saggital
797E	76%	80%	76%	82%	42%	55%	0	3	Rfc
797F	54%	47%	44%	61%	47%	54%	3	2	Rfc
797G	78%	82%	67%	94%	82%	97%	0	0	NA
Mean (SD)	66.9 (11)	61.9 (22)	57 (12)	74.4 (15)	42.6 (20)	58.1 (19)			

Note: - Ratio measures, mean (SD), and ventricular dilatation were assessed as described in Table 8.
- Transplant (Tp) size and location were assessed as described in Table 9.

Table 10.--Detailed morphometric ratio in various structures assessed of individual animals in the group sacrificed 3-4 weeks post-transplantation. N=11

Animal	Ant ctx	Post ctx	C - Pu	B stem	CA field	Dent gyrus	V dil	Tp size	Tp location
797H	73%	81%	51%	84%	34%	53%	1	1	Rfc
797I	60%	63%	38%	69%	47%	60%	0	3	Rfc
797J	114%	114%	99%	89%	62%	69%	3	3	lateral V
797K	74%	78%	66%	73%	30%	40%	1	3	Rfc
811A	60%	65%	48%	76%	52%	70%	0	2	Rfc
811B	62%	66%	57%	83%	0%	0%	1	3	Rfc
936D	57%	37%	45%	49%	72%	48%	2	3	Rfc
936E	86%	85%	83%	81%	40%	29%	0	2	Rfc
936F	78%	91%	58%	84%	83%	73%	0	0	NA
935A	57%	33%	58%	41%	23%	21%	2	0	NA
937B	35%	26%	22%	55%	11%	0%	3	0	NA
Mean (SD)	68.7 (20)	67.2 (27)	56.8 (21)	71.3(16)	41.3 (25)	42.1 (27)			

Note: - Ratio measures, mean (SD), and ventricular dilatation were assessed as described in Table 8.
- Transplant (Tp) size and location were assessed as described in Table 9.

Table 11.--Detailed morphometric ratio in various structures assessed of individual animals in the group sacrificed 6 weeks post-transplantation. N=8

Animal	Ant ctx	Post ctx	C-Pu	B stem	CA field	Dent gyrus	V dil	Tp size	Tp location
811C	39%	19%	15%	33%	0%	0%	3	3	Rfc
811D	78%	87%	70%	85%	60%	55%	0	3	Rfc
811E	57%	74%	0%	94%	72%	59%	1	0	NA
811F	79%	76%	65%	87%	38%	43%	0	1	Rfc
811G	95%	92%	88%	99%	72%	91%	0	1	Rfc
811H	90%	97%	79%	98%	64%	73%	0	3	Rfc
811I	91%	90%	97%	88%	89%	79%	0	2	ant rfc
811J	56%	64%	40%	67%	19%	22%	1	3	Rfc
Mean (SD)	73.1 (20)	74.9 (25)	56.8 (35)	81.4 (22)	51.8 (30)	52.8 (30)			

Note: - Ratio measures, mean (SD), and ventricular dilatation were assessed as described in Table 8.
- Transplant (Tp) size and location were assessed as described in Table 9.

APPENDIX B

Table 12.--Types and results of statistical analysis of the morphometric measures and the subjective score in study animals grouped by Tp size and compared to control animals receiving HI (III) only.

Structure assessed	Morphometric ratio measures (ANOVAI)	Subjective scoring system (Kruskal-Wallis test)
Anterior cortex	F= 0.26, P= 0.776	H= 0.053 with 2 df, P= 0.974
Posterior cortex	F= 0.06, P= 0.94	H= 0.284 with 2 df, P= 0.868
Caudate & Putamen	F= 0.77, P= 0.474	H= 0.553 with 2 df, P= 0.758
Brain Stem / Thalamus	F= 0.35, P= 0.705	H= 1.294 with 2 df, P= 0.524
CA fields	F= 0.72, P= 0.496	H= 1.529 with 2 df, P= 0.466
Dentate gyrus	F= 0.37, P= 0.692	H= 2.534 with 2 df, P= 0.282

Note: Analysis of variance for independent measures (ANOVI)
- Results show no siginificant difference (NS), either by ANOVAI for ratio measures, or Kruskal-Wallis test for the subjective scoring system.

Table 13.-- Types and results of statistical analysis of the morphometric measures and the subjective score in study animals grouped by age at sacrifice and compared to control animals receiving HI (III) only.

Structure assessed	Morphometric ratio measures (ANOVAI)	Subjective scoring system (Kruskal-Wallis test)
Anterior cortex	F= 0.0.31, P= 0.821	H= 2.398 with 2 df, P= 0.674
Posterior cortex	F= 0.33, P= 0.803	H= 3.635 with 3 df, P= 0.409
Caudate & Putamen	F= 0.32, P= 0.808	H= 0.778 with 3 df, P= 1.00
Brain Stem / Thalamus	F= 0.49, P= 0.688	H= 3.076 with 3 df, P= 0.515
CA fields	F= 0.63, P= 0.600	H= 1.376 with 3 df, P= 0.973
Dentate gyrus	F= 0.68, P= 0.568	H= 0.985 with 3 df, P= 1.00

Note: NS difference at any structure assessed.

Table 14.-- Correlation between the semi-quantitative damage assessment scoring system, and the ratio measures of all animals surviving HI according to treatment III (N=34) at the various structures assessed.

Structure Assessed	Correlation
Anterior Cortex	r = -0.855, df = 32, t = -9.338
Posterior Cortex	r = -0.739, df = 32, t = -6.205
Caudate & Putamen	r = -0.713, df = 32, t = -5.75
B stem / Thalamus	r = -0.888, df = 32, t = -10.925
CA Field	r = -0.714, df = 32, t = -5.771
Dentate Gyrus	r = -0.843, df = 32, t = -8.855
Overall Correlation	r = -0.775, df = 198, t = -17.27

Note: - Good negative correlation were observed at all structures, and a significant overall correalation.

APPENDIX C

Plots of the ratio measures for animals surviving treatment III HI at various structures assessed are shown on the next pages Figs. 22-27, at each structure animals were grouped by transplant size {as described earlier good (size 2-3), poor / no (size 0-1)} shown as the top plot of every page (Fig. a). The bottom plot of every page (Fig. b) is for the same set of animals but grouped by age at sacrifice, 2, 3-4, 6 wks post-transplantation and compared with animals sustaining HI only and sacrificed at 4 wks post HI insult. Arrows indicate the mean ratio of each group and the standard deviation (SD).

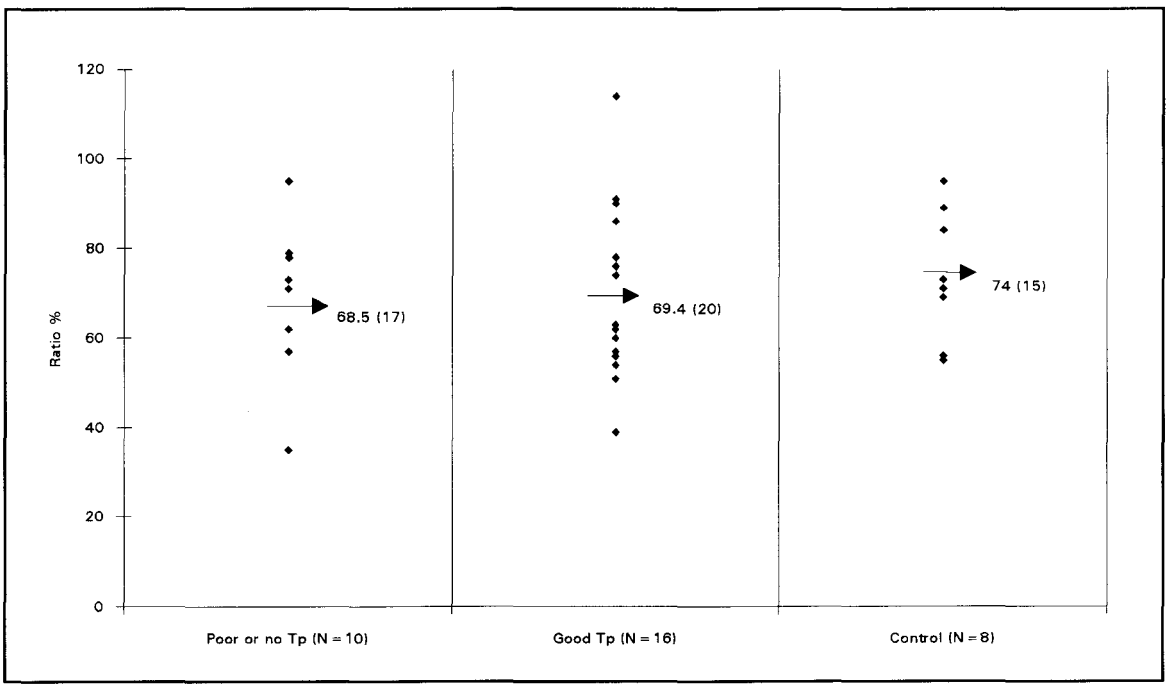


Figure 22a. Anterior hemisphere area ratio in animals grouped by Tp size.

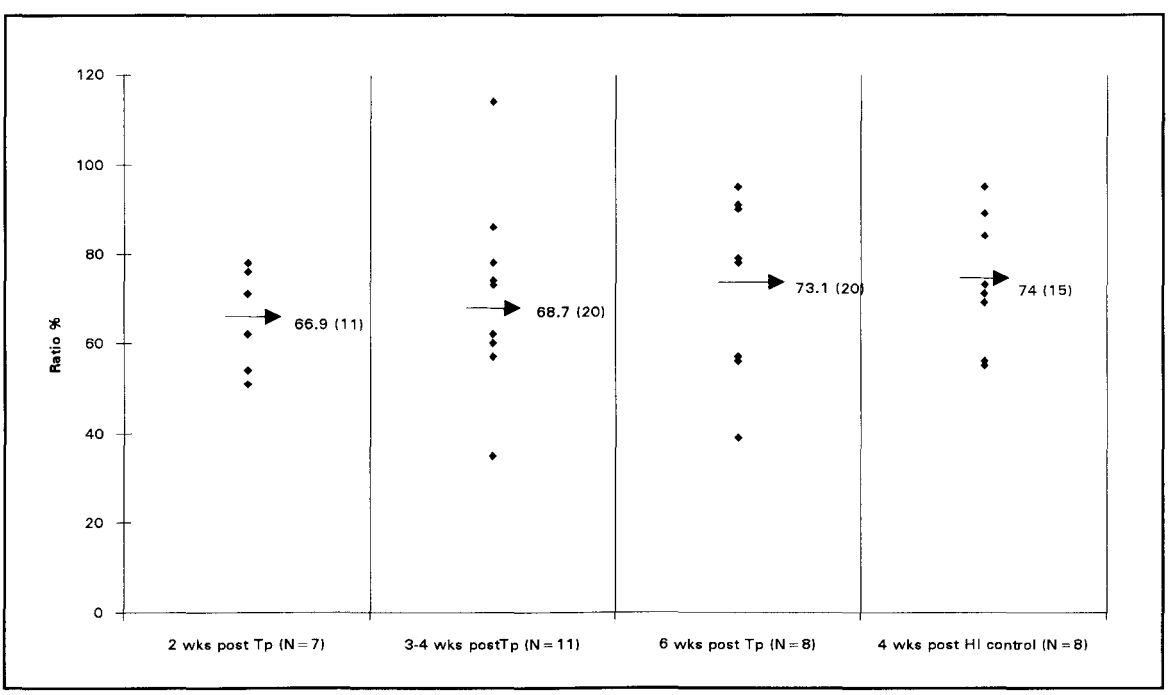


Figure 22b. Anterior hemisphere area measures in animals grouped by age at sacrifice.

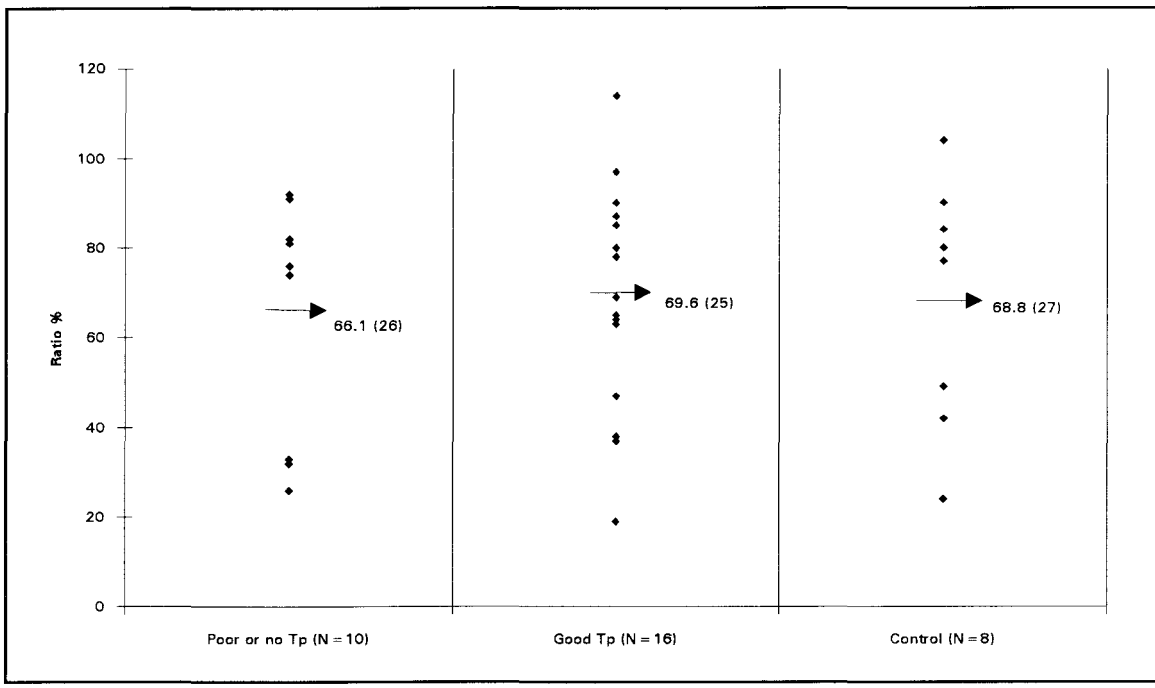


Figure 23a. Posterior hemisphere area ratio in animals grouped by Tp size.

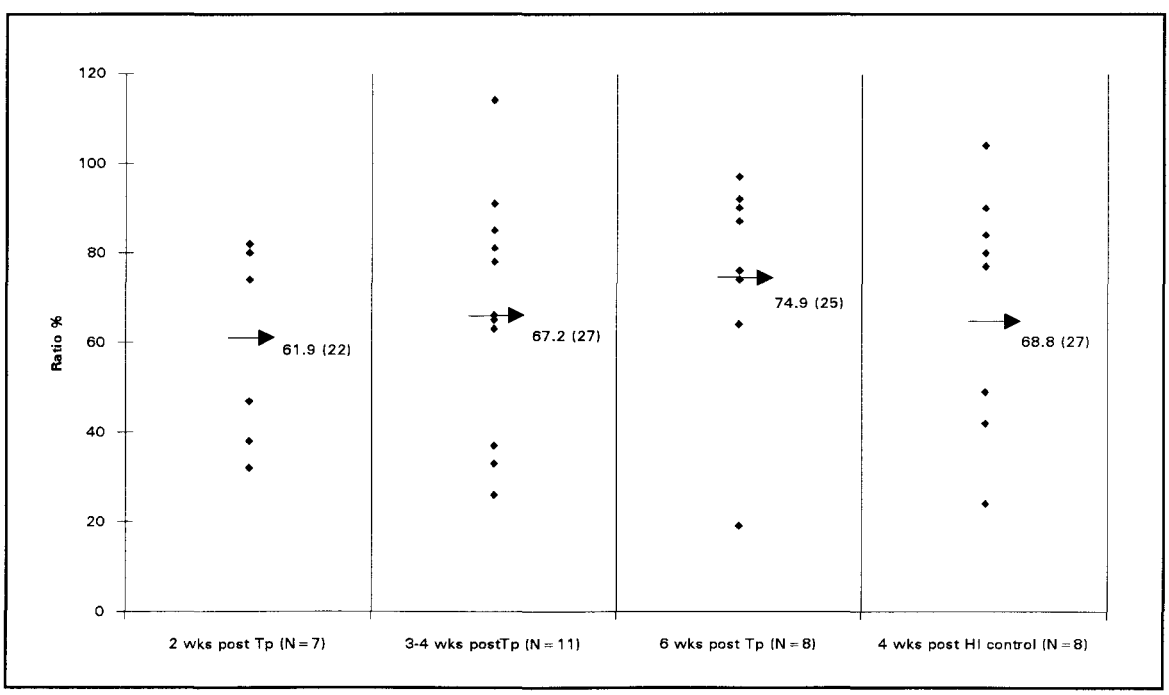


Figure 23b. Posterior hemisphere area ratio in animals grouped by age at sacrifice.

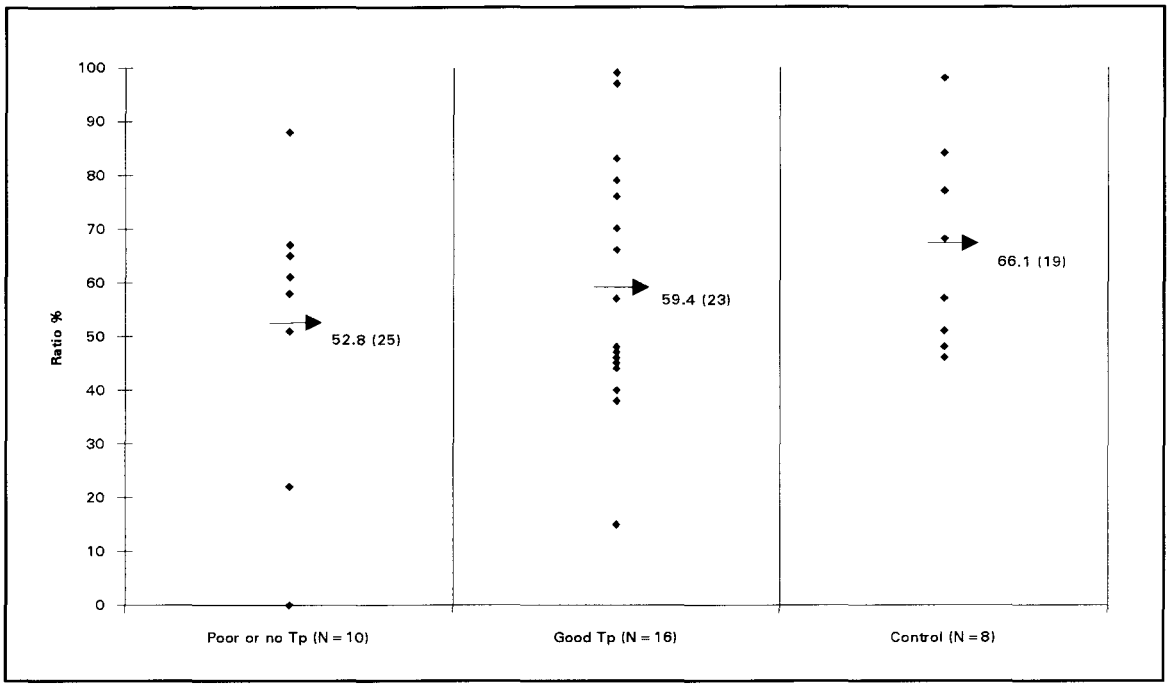


Figure 24a. Caudate & Putamen area ratio in animals grouped by Tp size.

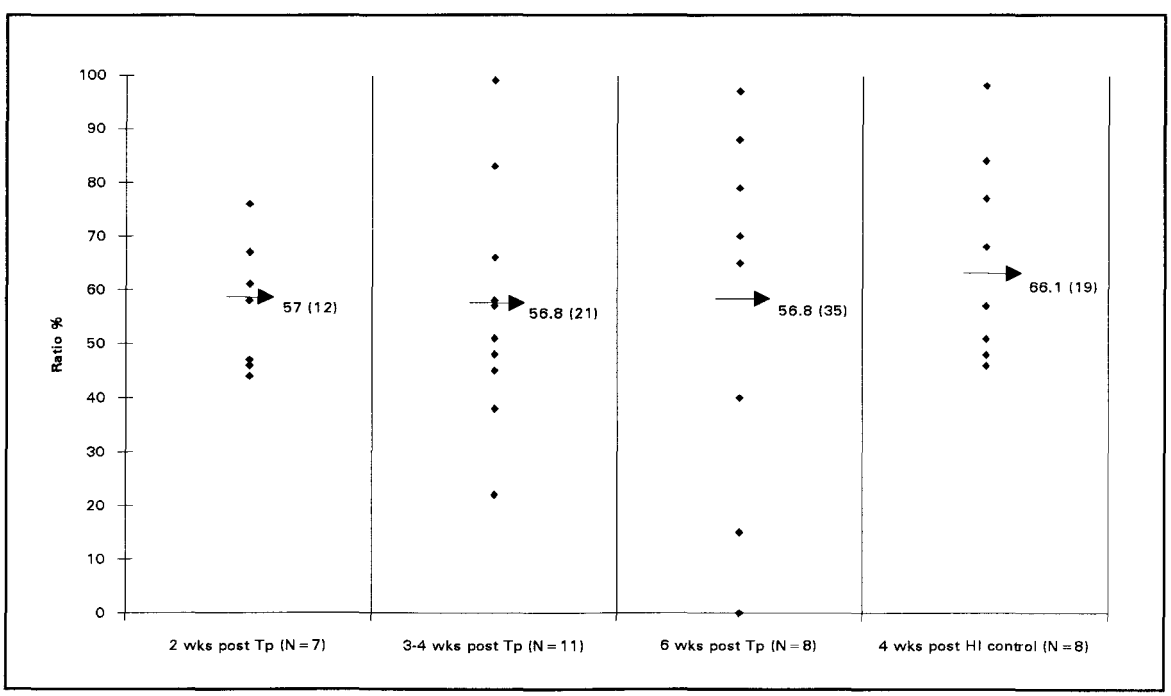


Figure 24b. Caudate & Putamen area ratio in animals grouped by age at sacrifice.

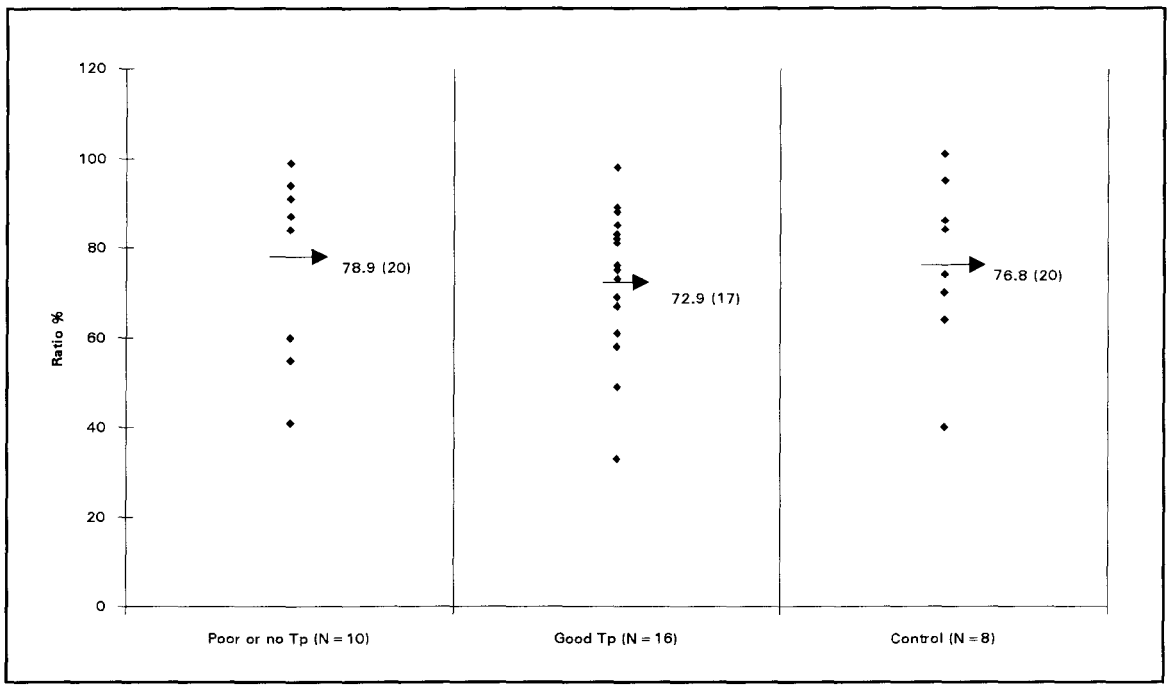


Figure 25a. Brain stem diencephalon area ratio for animals grouped by size of Tp.

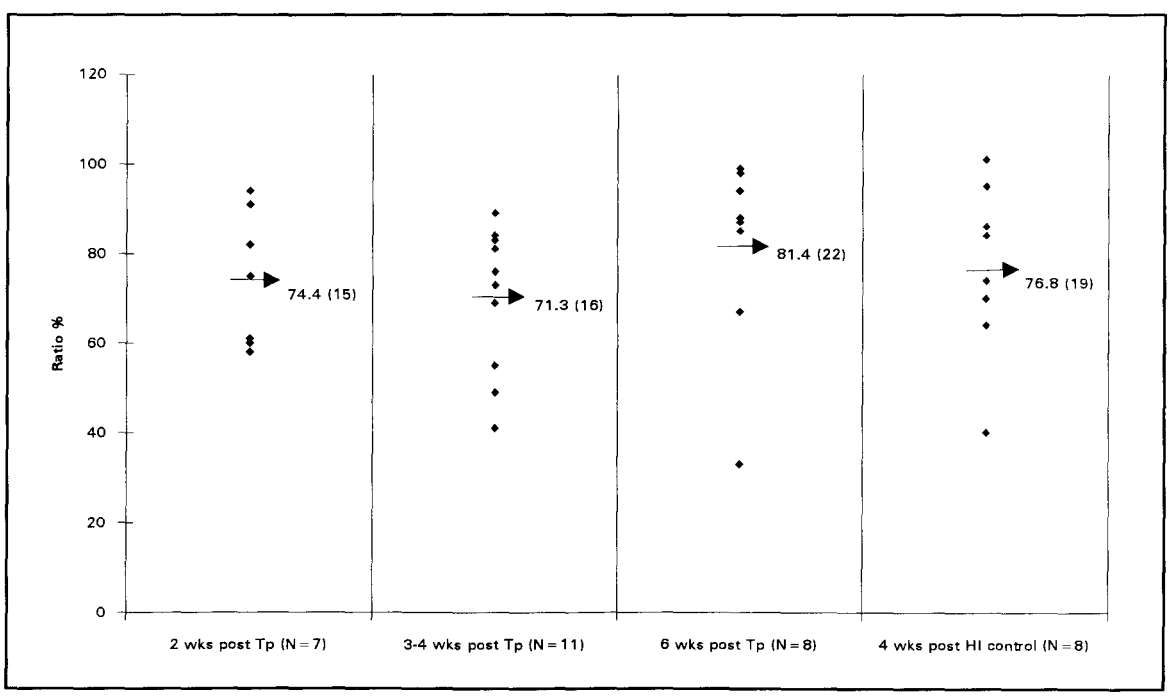


Figure 25b. Brain stem diencephalon area ratio in animals grouped by age at sacrifice.

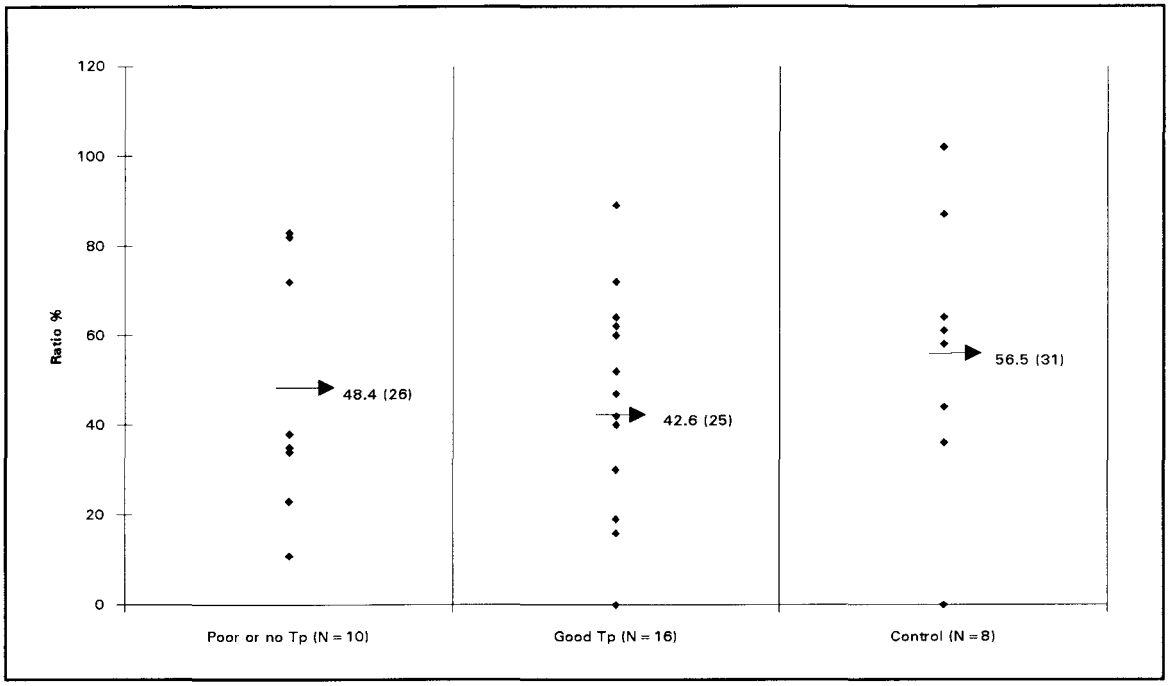


Figure 26a. CA field length ratio measures in animals grouped by Tp size.

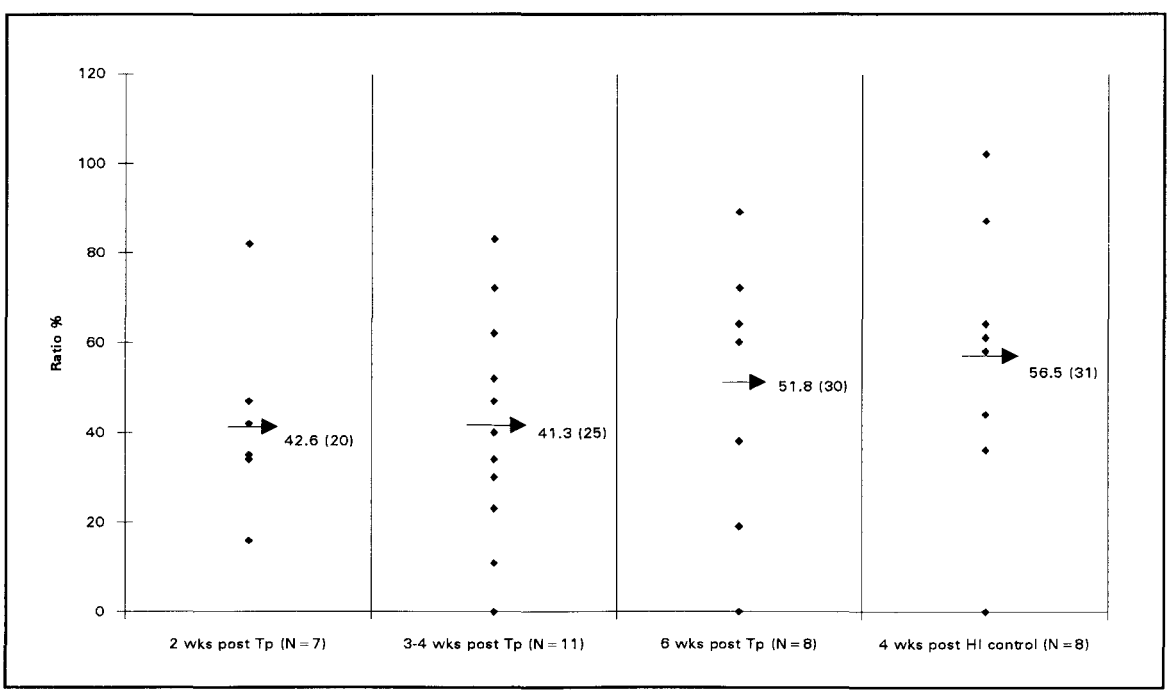


Figure 26b. CA field length ratio for animals grouped by age at sacrifice.

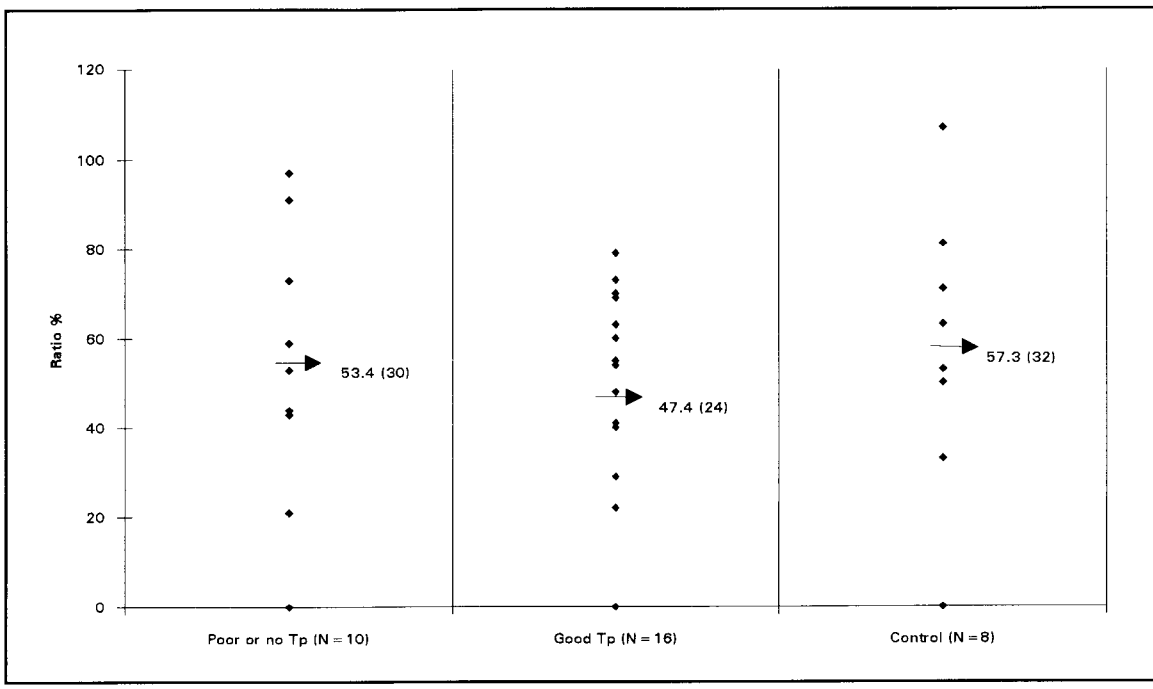


Figure 27a. Dentate gyrus length ratio for animals grouped by Tp size.

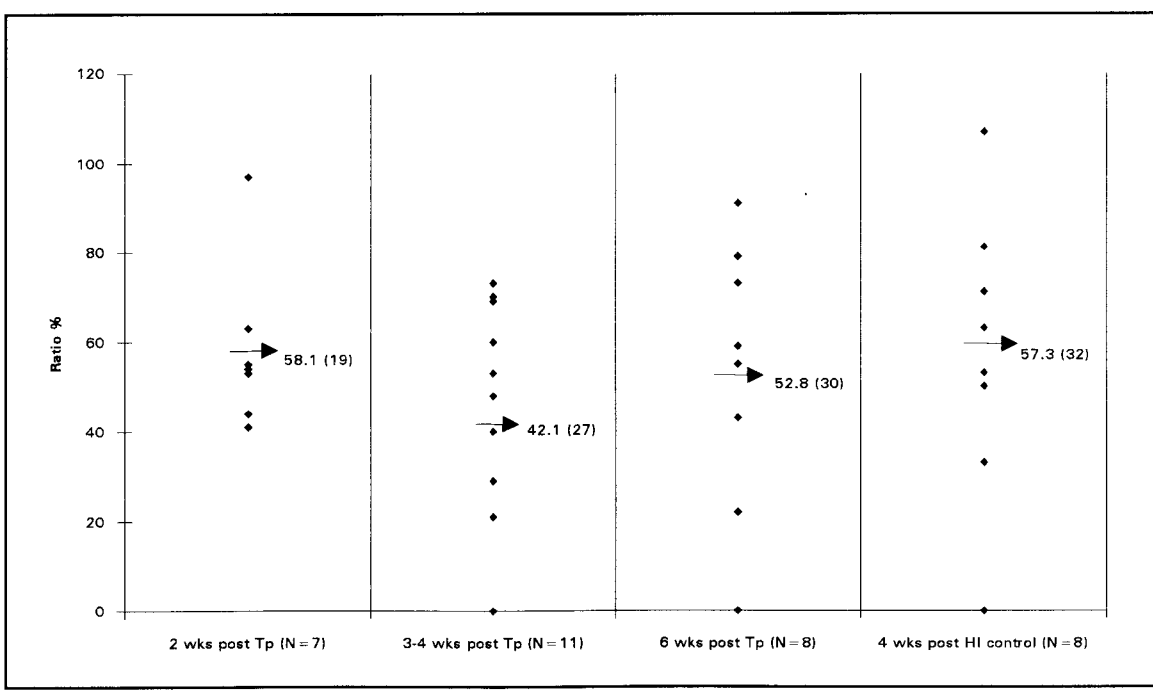


Figure 27b. Dentate gyrus length ratio in animals grouped by age at sacrificed.

REFERENCES

Andine P, Lehman A, Ellren K, et al. (1988) The excitatory amino acid antagonist kynurenic acid administered after hypoxic-ischemia in neonatal rats offers neuroprotection. Neurosci.Lett, 90:208-12

Backlund EO, Granberg PO, Hamberger B, Gedvall G. Olsen L. (1985) Transplantation of adrenal medullary tissue to striatum in parkinsonism. first clinical trials. J. Neurosurg, 62:169-73

Barker CF, Billingham RE. (1977) Immunologically privileged sites. Adv. Immunol, 25:1-25

Barks JDE, Silverstein FS, Sims K, Greenamyre JT, Johnston MV. (1988) Glutamaye recognition sites in human fetal brain. Neurosci Lett, 84:131-6

Barks JDE, Post M, Tuor UI. (1991) Dexamethasone prevents hypoxic-ischemic brain damage in the neonatal rat. Pediatr. Res, 29:558-563

Barks JDE, Silverstein FS. (1992) Excitatory amino acids contribute to the pathogenesis of perinatal hypoxic-ischemic brain injury. Brain Pathol, 2:235-243

Benveniste H, Drejer J, Schousboe A, Diemer NH. (1984) Elevation of the extracellular concentration of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. J Neurochem, 4:1369-74

Bjorklund A, Stenevi U, Dunnett SB, Gage FH. (1982) Cross species neural grafting in a rat model of Parkinson's Disease. Nature, 298:652-4

Blair E, Stanely FJ. (1988) Intrapartum asphyxia: A rare cause of cerebral palsy. J Peds, 112:215-20

Brann AW, Myers RE. (1975) Central nervous system findings in the newborn monkey following severe in utero partial asphyxia. Neurology, 25:327-38

Brown JK, Puruis RJ, Forfar JO. (1974) Neurologic aspects of perinatal asphyxia. Dev Med Child Neurol, 16:567

Burke RE, Kenyon N. (1991) The effect of neonatal hypoxia ischemia on striatal cholinergic neuropil - A quantitative morphologic analysis. Exp Neurol, 113:63-73

Castro AJ, Zimmer J, Sunde NA, Bold EL. (1985) Transplantation of fetal cortex to the brain of newborn rats: a retrograde fluorescent analysis of callosal and thalamic projections from transplant to host. Neurosci Lett, 60:283-288

Castro AJ, Tonder N, Sunde NA, Zimmer J. (1987) Fetal cortical transplants in the cerebral hemisphere of newborn rats. A retrograde fluorescent analysis of connections. Exp Brain Res, 66:533-42

Castro AJ, Tonder N, Sunde NA, Zimmer J. (1988) Fetal neocortical transplants grafted to the cerebral cortex of newborn rats receive afferents from the basal forebrain, locus coeruleus and midline raphe. Exp Brain Res, 69:613-22

Castro AJ, Sorensen JC, Tonder N, Bold L, Zimmer J. (1989) Fetal neocortical transplants grafted into cortical lesion cavities made in newborn rats receive multiple host afferents. A retrograde fluorescent tracer analysis. Restor Neurol Neurosci, 1:13-23

Castro AJ, Hogan TP, Sorensen JC, Klausen BS, Danielsen EH, Zimmer J, Neafsey EJ. (1991) Heterotopic neocortical transplants. An anatomical and electrophysiological analysis of host projections to occipital cortical grafts placed into sensorimotor cortical lesions made in newborn rats. Dev Brain Res, 58:231-36

Chang FL, Steedman JG, Lund RD. Embryonic cerebral cortex placed in the occipital region of newborn rats makes connections with the host brain. Dev Brain Res, 13:164-66

Chang FL, Steedman JG, Lund RD. (1986) The lamination and connectivity of embryonic cerebral cortex transplanted into newborn rat cortex. J Comp Neurol, 244:401-11

Choi DW. (1987) Dextrorphan and dextromethorphan attenuate glutamate neurotoxicity. Brain Res, 403:333-6

Choi DW. (1988a) Glutamate neurotoxicity and diseases of the nervous system. Neuron, 1:623-34

Choi DW, Koh JY, Peters S. (1988b) Pharmacology of glutamate neurotoxicity in cortical cell culture: Attenuation by NMDA antagonist. J Neurosci, 8:185-96

Chumas PD, Del. Bigio MR, Drake JM, Tuor UI. (1993) A comparison of the protective effect of dexamethazone to other potential prophylactic agents in a neonatal rat model of cerebral hypoxia-ischemia. J Neurosurg, 79:414-420

Clark GD, Rothman SM. (1987) Blockade of excitatory amino acid receptors protects anoxic hippocampal slices. Neuroscience, 21:665-71

Coacher A, Zivin JA, Lyden PD, Mazzarella V. (1988) Glutamate antagonist therapy reduces neurologic deficits produced by focal central nervous system ischemia. Arch Neurol, 45:148-53

Committee on Obstetrics, Maternal and Fetal Medicine. (1992) Fetal and neonatal neurologic injury. Technical Bulletin 163, Washington DC, American College of Obstetric and Gynecology, January

Das GD. (1974) Transplantation of embryonic neural tissue in the mammalian brain. Growth and differentiation of neuroblasts from various regions of the embryonic brain in the cerebellum of neonatal rats. J of Life Sci, 93-124

Das GD, Hallas HB. (1978) Transplantation of brain tissue in the brain of adult rat. Experimentia, 34:1304-1306

Dietrich WD. (1992) The importance of brain temperature in cerebral injury. J Neurotrauma, 9:S475-85

Duffy TE, Cavzzuti M, Cruz NF, Sokoloff L. (1982) Local cerebral glucose metabolism in newborn dogs: Effects of hypoxia and halothane anesthesia. Ann Neurol, 11:233-46

Dunn EH. (1917) Primary and secondary findings in a series of attempts to transplant cerebral cortex in albino rat. J Comp neurol, 271:565-582

Duverger D, MacKenzie ET. (1988) The quantification of cerebral infarction following focal ischemia in the rat: influence of strain, arterial pressure, blood glucose concentration, and age. J Cereb Blood Flow Metab, 8:449-461

Ellenberg JH, Nelson KB. (1988) Cluster of perinatal events identifying infants at high risk for death or disability. J Pediatr, 113 :546-552

Engelsen B. Neurotransmitter glutamate: Its clinical importance. (1986) Acta Neurol Scand, 74:337-55

Erselius RT, Wree A. (1991) Ultrastructure of axons in stereotaxically placed ibotenic acid-induced lesions of the hippocampus in adult rat - evidence for demyelination and

degeneration of dispersed axons of passage. J Hirnforsch, 32:139-148

Floeter MK, Jones EG. (1984) Connections made by transplants to the cerebral cortex of rat brain damaged in utero. J Neurosci, 4;141-150

Floeter MK, Jones E. (1985) Transplantation of fetal postmitotic neurons to rat cortex: Survival, early pathway choices and long-term projections of outgrowing axons. Dev Brain Res, 22:19-38

Fonseca MJ, DeFilipe, Fairen A. (1988) Local connections in transplanted normal cerebral cortex of rats. Exp Brain Res, 69:387-398

Ford LM, Sanberg PR, Norman AB, Fogelson MH. (1989) MK-801 prevents hippocampal neurodegeneration in neonatal hypoxic-ischemic rats. Arch Neurol, 46:1090-96

Freeman JM (ed). (1985) Prenatal and perinatal factors associated with brain disorders. Reports to NICHHD, NIH Publication No. 85-1149, Washington DC, US Government Printing Office

Freeman JM, Nelson KB. (1988) Intrapartum asphyxia and cerebral palsy. Pediatrics, 82:240-249

Genser-Jensen FA, Blackstad TW. (1971) Distribution of acetylcholinesterase in the hippocampal region of the guinea pig. Z zellfforsch, 114:460-81

Gibbs RB, Cotman CW. (1987) Factors affecting survival and outgrowth from transplants of entorhinal cortex. Neuroscience, 21:699-706

Gonzalez MF, Sharp FR. (1987) Fetal frontal cortex transplanted to injured motor/sensory cortex of adult rats. I.NADPH-diaphorase neurons. J Neurosci, 7:2991-3001

Gonzalez MF, Sharp FR, Loken JE. (1988) Fetal frontal cortex transplanted to injured motor/sensory cortex of adult rats: Reciprocal connections with host thalamus demonstrated with WGA-HRP. Exp Neurol, 99:154-65

Grabowski M, Brundin P, Johansson BB. (1992) Fetal neocortical grafts implanted in adult hypertensive rats with cortical infarcts following a middle cerebral artery occlusion: Ingrowth of afferent fibers from the host brain. Exp Neurol, 116:105-121

Greenamyre JT, Penney JB, Young AB, Hudson C, Silverstein FS, Johnston MV. (1987) Evidence for transient perinatal glutamatergic innervation of globus pallidus. J Neurosci, 7:1022-30

Hadani M, Freeman T, Pearson J, Young W. Flamm E. (1987) Embryonic cortical transplants survive in middle cerebral artery territory after permanent arterial occlusion in adult rats. Ann N.Y. Acad. Sci, 495:711-14

Hagberg H, Anderson P, Kjellmer I. (1987) Extracellular overflow of glutamate, aspartate, GABA and taurine in the cortex and basal ganglia of fetal lambs during hypoxia-ischemia. Neurosci Lett, 78:311-7

Hattori H, Wasterlian CG. (1990) Post-hypoxic glucose supplement reduces hypoxic-ischemic brain damage in the neonatal rat. Ann Neurol, 28:122-128

Hedreen JC, Bacon SJ, Price D. (1985) A modified histochemical technique to visualize acetylcholinesterase-containing axons. J Histochem Cytochem, 33:134-40

Hitchcock ER. (1989) Recent experiences with dopamine transplantation for Parkinson's Disease, in Proceedings of Society of British Neurological Surgeons, Oxford. J Neurosurg Psychiat, 52:141

Hohmann CF, Ebner FF. (1988) Basal forebrain lesions facilitate adult host fiber ingrowth into neocortical transplants. Brain Res, 448:53-66

Huan F, Cunningham TJ. (1984) Cortical transplants reveal CNS trophic interactions in situ. Dev Brain Res, 15:290-4

Huan F, Cunningham TJ. (1987) Specific neurotrophic interactions between cortical and subcortical visual structures in developing rat: in vivo studies. J Comp Neurol, 256:561-69

Ikonomidou C, Price MT, Mosinger JL, Frirdich J, Labruyere J, Shallid Sallas K, Olney JW. (1989a) Hypobaric-ischemic conditions produce glutamate-like cytopathology in infant brain. J Neurosci, 9:1693-1700

Ikonomidou C, Mosinger JL, Shallid Sallas K, Labruere J, Olney JW. (1989b) Sensitivity of the developing rat brain to hypobaric/ischemic damage parallels sensitivity to N-Methyl-Aspartate neurotoxicity. J Neurosci, 9:2809-18

Izumiyama K, Kogure K. (1988) Prevention of delayed neuronal death in gerbil hippocampus by calcium-ion channel blockers. Stroke, 19:1003-7

Johnson MA, Pennock JM, Bidder G, Debase LAS, Thomas DJ, Young I. (1987) Serial MR imaging in neonatal cerebral injury. AJAR, 8:83-92

Konishi Y, Kuriyama M, Sudo M, Hayakawa K, Ishii Y. (1987) Superior sagittal sinus thrombosis in neonates. Pediatr Neurol, 3:222-5

Le Gross Clarke WE. (1940) Neuronal differentiation in implanted foetal cortical tissue. J Neurol Psychiat, 3:262-72

Lee MH, Rabe A, Currie JR, Shek J, Wisniewski HM. (1987) Transplants of normal fetal cerebral cortical tissue into congenitally malformed brains of infant rats. Ann N.Y. Acad Sci, 495:732-35

Levene MI, Kornberg J, Williams THC. (1985) The incidence and severity of post-asphyxial encephalopathy in full-term infants. Early Human Development, 11:21

Levene MI, Sands C, Grindulis H, Moore JR. (1986) Comparison of two methods of predicting outcome in perinatal asphyxia. Lancet, i:67-8

Levine S. (1960) Anoxic-ischemic encephalopathy in rats. Am J Pathol, 36:1-17

Lou HC, Lassen NA, Tweed WA, Johnson G, Jones M, Palahniuk RJ. (1979a) Pressure-Passive cerebral blood flow and breakdown of the blood-brain barrier in experimental fetal asphyxia. Acta Paediatr Scand, 68:35-44

Lou HC, Lassen NA, Friis-Hansen B. (1979b) Impaired autoregulation of cerebral blood flow in the distressed newborn. J Pediatr, 94:118-25

Lou HC. (1988) the "lost autoregulation hypothesis" and brain lesions in the newborn-An update. Brain Dev, 10:143-6

Low JA. (1988) Fetal asphyxia in the antepartum and intrapartum period. Can Med Assoc J, (Suppl):49-53

Lund RD, Hauschka SD. (1976) Transplanted neural tissue develops connections with host rat brain. Science, 193:582-84

Madrazo I, Leon V, Torres C, Aguilera MJC, Valera G, Alvarez F, Fraga A, Colin-Drucker R, Ostrosky F, Skurorich M, Franco R. (1988) Transplantation of foetal substantia nigra and adrenal medulla to the caudate nucleus in two patients with Parkinson's Disease. New Eng J Med, 318:51

Mampalan TJ, Gonzalez MF, Weinstein P, Sharp FR. (1988) Neuronal changes in fetal cortex transplanted to ischemic adult rat cortex. J Neurosurg, 69:904-12

McArdle CB, Richardson CJ, Hayden CK, Nicholas DA, Amparo EG. (1987) Abnormalities of the neonatal brain: MR imaging,part II.hypoxic-ischemic brain injury. Radiology, 163:395-403

MacDonald HM, Mulligan JC, Allen AC, Taylor PM. (1980) Neonatal asphyxia,

I:relationship of obstetric and neonatal complications to neonatal mortality in 38,405 consecutive deliveries. J Pediatr, 96:898-902

McDonald JW, Silverstein FS, Johnston MV. (1987) MK-801 protects the neonatal brain from hypoxic-ischemic damage. Eur J Pharmacol, 140:359-61

McDonald JW, Silverstein FS, Johnston MV. (1988) Neurotoxicity of N-Methyl-D-Aspartate is markedly enhanced in developing rat central nervous system. Brain Res, 459:200-203

McDonald JW, Johnston MV. (1990) Pharmacology of N-Methyl-D-Aspartate induced brain injury in an in vivo perinatal brain model. Synapse, 6:179-188

McDonald JW, Chen CK, Trescher WH, and Hohnston MV. (1991) The severity of excitotoxic brain injury is dependent on brain temperature in immature rat. Neurosci Lett, 126:83-86

Mufson EJ, Labbe R, Stein DG. (1987) Morphologic features of embryonic neocortex grafts in adult rats following frontal cortical ablation. Brain Res, 401:162-7

Mujsce DJ, Towfighi J, Stern D, Vannucci RC. (1990a) Mannitol therapy in perinatal hypoxic-ischemic brain damage in rats. Stroke, 21:1210-14

Mujsce DJ, Christensen MA, Vannucci RC. (1990b) Cerebral blood flow and edema in perinatal hypoxic-ischemic brain damage. Pediatr Res, 27:450-53

Mulligan JC, Painter MJ, O'Donoughue PA. (1980) Neonatal asphyxia,II: neonatal mortality and long-term sequelae. J Pediatr, 96:903-907

Myers RE. (1969a) Brain pathology following fetal vascular occlusion: An experimental study. Invest Ophthalmol, 8:41

Myers RE, Beard R, Adamsons K. (1969b) Brain swelling in the newborn rhesus monkey following prolonged partial asphyxia. Neurology, 19:1012-18

Myers RE. (1972) Two patterns of perinatal brain damage and their conditions of occurrence. Amer J Obst Gyn, 26:21

Myers RE. (1975) Fetal asphyxia due to umbilical cord compression. Biol Neonate, 26:21-43

Naeye RL, Peters EC, Bartholomew M, et al. (1989) Origin of cerebral palsy. Am J Dis Child, 143:1154-61

Neafsey EJ, Sorensen JC, Tonder N, Castro AJ. (1989) Fetal cortical transplants into neonatal rats respond to thalamic peripheral stimulation in the adult. An electrophysiological study of single-unit activity. Brain Res, 493:33-40

Nelson KB, Ellenberg JH. (1986) Antecedents of cerebral palsy : Multivariate analysis of risk. N Engl J Med, 315:81-6

Nieto-Sampedro M, Lewis ER, Cotman CW, Manthorpe M, Skaper SD, Barbin G, Longo FM, Varon S. (1982) Brain injury causes a time-dependent increase in neuronotrophic activity at the lesion site. Science, 217:860-61

Nieto-Sampedro M, Manthrope M, Barbin G, Varon S, Cotman CW. (1983) Injury-induced neuronotrophic activity in adult rat brain: correlation with survival of delayed implants in the wound cavity. J Neurosci, 3:2219-29

Nieto-Sampedro M, Bovolenta P. (1990) Growth factors and growth factor receptors in the hippocampus : Role in plasticity and response to injury. Prog. Brain Res, Storm-mathisen and zimmer ed.

Olney JW. (1978) Neurotoxicity of excitatory amino acids. In Mcgeer EG, et al (eds): Kainic Acid as a Tool in Neurobiology. New York, Raven Press, pp 95-121

Palmer C, Vannucci RC, and Towfighi J. (1990) Reduction of perinatal hypoxic-ischemic brain damage with Allopurinol. Peds Res, 27:332-336

Paxinos & Charles. (1986) The Rat Brain in Stereotaxic Coordinates. 2 nd editition. Academic Press, New York

Petito CK, Feldman E, plum F. (1987) Delayed hippocampal damage in humans following cardiorespiratory arrest. Neurology, 37:1281-6

Porter LL, Cederbaum JM, O'leary DDM, Stanfield BB, Asanuma H. (1987) The physiologic identification of pyramidal tract neurons within transplants in the rostral cortex taken from the occipital cortex during development. Brain Res, 436:136-42

Pulsinelli WA, Brierley JB, Plum F. (1982) Temporal profile of neuronal damage in a model of a transient forebrain ischemia. Ann Neurol, 11:491-9

Rice JE, Vannucci RC, Brierley JB. (1981) The influence of immaturity on hypoxic-ischemic brain damage in the rat. Ann Neurol, 9;131-41

Robertson CM, Finer N. (1985) Term infants with hypoxic-ischemic encephalopathy: outcome at 3.5 years. Dev Med Child Neurol, 27:473-484

Robertson CM, Finer N., and Grace M.G. (1989) School performance of survivors of neonatal encephalopathy associated with birth asphyxia at term. J Pediatr, 114:753

Ronald EH, Hill A, Norman MG, Flodmark O, Macnab AJ. (1988) Selective brainstem injury in an asphyxiated newborn. Ann Neurol, 23:89-92

Ross DT, Ebner FF. (1990) Thalamic retrograde degeneration following cortical injury: an excitotoxic process?. Neurosci, 35:525-550

Rothman SM. (1983) Synaptic activity mediates death of hypoxic neurons. Science, 220:536-7

Rothman SM, Thurston Jh, Hauhart RE, Clark Gd, Solomon Js. (1987) Ketamine protects hippocampal neurons from anoxia in vitro. Neuroscience, 21:673-8

Santacana M, Heredia M, Valverde F. (1990) Transplant connectivity in the rats cerebral cortex. A carbocyanine study. Dev Brain Res, 56:217-22

Sharp F, Gonzalez M. (1986) Fetal cortical transplants ameliorate thalamic atrophy ipsilateral to neonatal frontal cortex lesions. Neurosci Lett, 71:247-51

Silverstein FS, Johnston MV. (1984) Effects of hypoxia-ischemia on monoamine metabolism in the immature brain. Ann Neurol, 15:342-7

Silverstein FS, Buchanan K, Johnston MV. (1986) Perinatal hypoxia-ischemia disrupts striatal high-affinity [3H] glutamate uptake into synaptosome. J Neurochem, 47:1614-9

Silverstein FS, Torke LY, Barks J, Johnston MV. (1987) Hypoxia-ischemia produces focal disruption of glutamate receptors in developing brain. Dev Brain Res, 34:33-9

Sladek JR, Gash DM. (1984) Neural transplants. Planum Press, New York

Sloan DJ, Wood MJ, Charlton HM. (1991) The immune response to intracerebral neural grafts. TINS, 14:341-46

Soares H, Mcintosh TK. (1991) Fetal cortical transplants in adult rats subjected to experimental brain injury. J Neural Transp Plast, 2;No.3-4:207-220

Sofroniew MV, Isacson O, Bjorklund A.(1986) Cortical grafts prevent atrophy of cholinergic basal nucleus neurons induced by excitotoxic cortical damage. Brain Res, 378:409-15

Sorensen J, Zimmer J, Castro AJ. (1989) Fetal cortical transplants reduce the thalamic atrophy induced by frontal cortical lesion in newborn rats. Neurosci Lett, 98:33-38

Sorensen JC, Wanner-Olsen H, Tonder N, Danielsen E, Castro AJ, Zimmer J. (1990) Axotomized, adult basal forebrain neurons can innervate fetal frontal cortex grafts: A double fluorescent tracer study in the rat. Exp Brain Res, 81:545-51

Sorensen JC, Castro AJ, Klausen B, Zimmer J. (1992) Projections from fetal neocortical transplants placed in the frontal neocortex of newborn rats. A phaseolus vulgaris-leucoagglutinin tracing study. Exp Brain Res, 92:299-309

Thirlinger K, Hrbek A, Karlsoon K, Rosen KG, Kjellmer I. (1987) Post-asphyxial cerebral survival in the newborn sheep after treatment with oxygen free radical scavengers and a calcium antagonist. Pediatr Res, 22:62-6

Thompson WG. (1890) Successful brain grafting. New York Med J, 51:701-2

Towfighi J, Yager JY, Housman C, Vannucci RC. (1991) Neuropathology of remote hypoxic-ischemic damage in the immature rat. Acta Neuropathol, 81:578-87

Vannucci RC, Christensen MA, Stein DT. (1989) Regional cerebral glucose utilization in the immature rat: effect of hypoxia-ischemia. Peds Res, 26:208-14

Vannuci RC. (1990) Current and potentially new management strategies for perinatal hypoxic-ischemic encephalopathy. Pediatrics, 85:961-968

Versmold HT, Kitterman JA, Phibbs RH, Gregory GA, Tooley WH. (1981) Aortic blood pressure during the first 12 hours of life in infants with birth weight 610 to 4220 grams. Pediatrics, 67:607-13

Volpe JJ. (1987) Hypoxic-ischemic encephalopathy. In: Neurology of the newborn. 2nd ed. Philadelphia: WB Saunders Co, 159-280

VITA

The author, M. Hany Elsayed, was born on the fifteenth of August 1957, in Cairo, Egypt. Hany attended Ain-Shams University School of Medicine in Cairo, where he received his M.D. degree in 1982 with grade: Very Good. After completing a rotating internship at Ain-Shams University Hospitals and serving the Ministry of Health in Egypt, he began his post-graduate medical training in the United States in 1986 when he started a Pediatric Residency at the College of Physician and surgeons of Columbia University at Harlem Hospital, New York. After completing his residency in 1989, he joined Loyola University and began a Fellowship in Neonatal-Perinatal Medicine. During his fellowship, he joined the Neuroscience Graduate Program at Loyola and began working in the laboratory of Dr. Anthony J. Castro in the department of Cell Biology, Neurobiology and Anatomy where he completed his Masters thesis work.

Dr. Elsayed has been on the Faculty of the Pediatric Department at Loyola as an Assistant Professor and working as an attending Neonatologist since completing his fellowship in 1992. He is board certified by the American Board of Pediatrics and the subboard of Neonatal-Perinatal Medicine.

APPROVAL SHEET

The thesis submitted by M. Hany Fathy Elsayed has been read and approved by the following committee :

Dr. Anthony J. Castro, Director
Professor
Department of Cell Biology, Neurobiology and Anatomy
Loyola University of Chicago

Dr. Edward J. Neafsey
Professor
Department of Cell Biology, Neurobilogy and Anatomy
Loyola University of Chicago

Dr. Thomas F. Myers
Associate Professor
Department of Pediatrics
Loyola University of Chicago

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and the thesis is now given final approval by the committee with reference to content and form.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

14 April 94
Date

Director's Signature