Adjunctive Drug Therapy for Treatment of Experimental Hydrocephalus

A thesis presented to the University of Manitoba
In partial fulfillment of requirements for the
Degree of Masters in Science in the
Department of Surgery

Presented by
Eric M. Massicotte, B.Sc., M.D.

May, 2000

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

COPYRIGHT PERMISSION PAGE

Adjunctive Drug Therapy for Treatment of Experimental Hydrocephalus

BY

Eric M. Massicotte

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of

Master of Science

Table of Contents

Acknowledgments

I would like to thank my supervisor, Dr. Marc Del Bigio. His guidance and support have made the completion of this manuscript possible.

Funding for the experiments came from several different sources – Manitoba Health Research Council, Health Sciences Centre Foundation, and the Frank E. Nulsen Hydrocephalus Research Fellowship. The University of Manitoba, Department of Surgery, generously provided the financial support to cover my tuition as a graduate student.

A special mention should be made for the excellent technical support provided by Cathy Crook and Qui Lan Wang. My thanks extend to Dr. Richard Buist for providing the MR images.

I dedicate this manuscript to my wife and daughter, Mary and Sara.

Abstract

The surgical treatment of hydrocephalus became commonplace almost four decades ago. Diversion of cerebrospinal fluid is, however, still accompanied by significant complications. However, the area of adjunctive drug therapy for treatment of hydrocephalus directly targeting neuro-protection and regeneration has not received much attention.

We investigated two drugs, nimodipine and AXOKINE ®, and their effect on functional outcome in an infant rat model of experimental hydrocephalus. The calcium channel blocker nimodipine may have neuro-protective properties by reducing the neuronal influx of calcium. AXOKINE ®, a second generation modified ciliary neurotrophic factor, could enhance regeneration of the neuronal population.

Hydrocephalus was achieved by injecting kaolin into the cisterna magna of three-week-old rats. Hydrocephalus was allowed to develop for two weeks prior to beginning treatment with nimodipine subcutaneously (1.8 mg per day) for 14 days at which time the rats were killed. The rats in this group were not surgically treated with shunting. In a separate experiment hydrocephalus was allowed to progress for three weeks. The rats were then shunted and intraventricular administration of a second generation ciliary neurotrophic factor, AXOKINE ® at either a low dose (1.4 μg/day) or high dose (14.3 μg/day) was started. Functional assessment consisted of motor, memory and learning components. Motor function was tested using spontaneous activity counting, ability to

stay on a rotating drum, and swimming. Memory and learning were quantitatively monitored using a modified Morris water maze. Histological examination provided pathological correlates to the functional outcome.

Nimodipine-treated rats exhibited significantly better motor performance, reducing the time required to swim 1.5 m by 27-47% compared to controls. Their superior agility was demonstrated by staying on a rotating drum 15-32% longer. The same group of rats had better memory and learning abilities. The swimming times required by the nimodipine-treated rats to find a platform on the first trial of the day improved by over 68% when compared to controls. Histological examination showed a thicker corpus callosum in nimodipine-treated rats.

Several factors confounded the AXOKINE ® experiment. Significant inter-group differences in mortality, change in ventricular size and weight gain were observed between the control and AXOKINE ®-treated animals. No obvious benefit of AXOKINE ® treatment could be detected.

Nimodipine seems to provide a certain degree of axonal protection, which correlated with a better functional outcome. Considering that this is a well tolerated drug, extension to investigation of human subjects may be promising. Investigation of other neurotrophic agents in the post-shunt period should be considered. Keeping in mind the incidence of hydrocephalus and the complications of CSF diversion the use of adjunctive drug therapy seems to have potential and warrants further attention.

List of Figures

List of Tables

List of Abbreviations

AIDS	Acquired immunodeficiency syndrome
ANOVA	Analysis of variance
B.I.D.	Twice a day
bFGF	Basic fibroblast growth factor
Ca^{2+}	Calcium
CBF	Cerebral blood flow
CC	Corpus callosum
CGalT	Ceramide galactosyltransferase
cm	Centimeter
CNPase	2',3'-cyclic nucleotide 3' phosphohydrolase
CNTF	Ciliary neurotrophic factor
CSF	Cerebrospinal fluid
CT	Computerized tomography
DMSO	Dimethylsulfoxide
GAP-43	Growth-associated protein-43 kD
GFAP	Glial fibrillary acidic protein
H&E	Hematoxylin and eosin
i.d.	Internal diameter

i.m.	Intramuscular
IGF-1	Insulin-like growth factor
Inc.	Incorporated
kD	Kilodalton
kg	kilogram
M	Mole
m	meter
MBP	Myelin basic protein
mg	milligram
ml	milliliter
mm	millimeter
MR	Magnetic resonance
MRI	Magnetic resonance imaging
Na^+	Sodium ion
NF	Neurofilament
NGF	Nerve growth factor
NMDA	N-methyl D-aspartate
o.d.	Outer diameter
O2A	Oligodendrocyte precursor
^oC	degree Celsius

PBS	Phosphate buffered saline
PDGF	Platelet derived growth factor
PEG	Poly-ethylene glycol
PO	per os
RNA	Ribonucleic acid
rpm	Revolution per minute
s.c.	Subcutaneous
S/C	Solochrome cyanine
T	Tesla
TE	Time between successive echoes
TR	Repetition time
VSCC	Voltage sensitive calcium channel
μm	micrometer

1.0 Review of the literature

1.1 Introduction

The pathophysiological definition of hydrocephalus is an imbalance of cerebrospinal fluid (CSF) formation and absorption leading to a net accumulation of CSF in the cerebral ventricles [104]. Hydrocephalus is second only to spina bifida as the most frequent congenital neurologic malformation in North America [159]. Surgical treatment of this accumulated CSF by diversion has been in use since 1929 [30]. The identification of silicone polymer as a superior biomaterial for the construction of tubes and valves provided neurosurgeons with the option of shunting excess CSF to other body cavities. This technique was first described in 1957 [123], and is still mainstay of hydrocephalus management today.

The wide variety of clinical presentations and tempo of progression of hydrocephalus can make timing of CSF diversion a challenge. Patients with raised intracranial pressure and ventriculomegaly do not present a management conundrum; however, not all cases demonstrate clear evidence of progression and some may even undergo regression or resolution. This challenge is compounded by the significant risk of failure associated with treatment. In a recent study, shunt failure occurred in 43.6% of 367 pediatric patients followed for a minimum of one

year [45]. The ultimate goal of minimizing tissue destruction caused by hydrocephalus through use of early shunting is counter-balanced by the significant risks associated with shunting. Drug therapy in the treatment of infantile hydrocephalus has focused on reducing intracranial hypertension through suppression of CSF production. The reduction of CSF production using osmotic diuretics has been used for many years [75]. Glycerol and isosorbide have been limited in their efficacy due to the dehydration they cause [66;90]. The use of less dehydrating agents like furosemide and acetazolamide has been advocated by some for reducing the need for CSF diversion [137]. However, in a recent international trial the same agents were associated with a higher mortality rate and greater number of patients requiring a shunt [1]. No drug therapies targeting neuronal protection or regeneration have been documented. The limited information regarding this area of hydrocephalus management has prompted this study.

1.2 Animal models of experimental hydrocephalus

Most information regarding pathophysiologic mechanisms of hydrocephalus is based on animal models. Many different animals including rats,

mice, kittens, cats, rabbits and dogs have been used experimentally. Materials injected into CSF pathways include bacteria, viruses, blood, lampblack, India ink, gelatin, balloons, silicone oil and kaolin [95;72] all of which can result in obstruction of CSF flow [71;73]. The difficulty encountered with many of these techniques is the unpredictable results [95]. The most common materials used at present are silicone and kaolin. Injection of kaolin into the cisterna magna produces a mild inflammatory response that obstructs CSF flow in the subarachnoid space and basal cisterns [95]. This model of hydrocephalus is most similar to post-meningitis hydrocephalus in human infants [113].

1.3 Behavioral assessment

Hydrocephalus is a progressive disorder of the brain with bilateral symmetry. Longitudinal data is required for assessing the effect of ventricular enlargement [39]. We know that hydrocephalus in humans can manifest itself in many ways. The most prominent motor disability is abnormal gait secondary to stretching of the corticospinal tract and effect on pre-motor areas[52;103]. Impaired cognitive development [146] and language disability [42], presumed to be the result of diffuse cortical dysfunction, have also been reported. Delayed growth and short stature have been attributed to the pressure on the hypothalamic area. Urinary

incontinence is frequently observed [79] due to loss of voluntary supraspinal control of micturition inducing hyperactivity and impairment of bladder sensation [2]. Rare signs and symptoms such as Parkinsonian movements [28], akinetic mutism [5] and paraparesis [162] have also been reported, possibly due to interruption of striatonigral and dopaminergic pathways.

Hydrocephalic rats have been shown to have learning disabilities [36;37;81]. Reports of impaired memory acquisition were reported in H-Tx rats, which have hereditary autosomal recessive aqueductal stenosis [65].

1.4 Pathological changes in hydrocephalus

The pathological changes secondary to hydrocephalus have been reviewed in detail [33;98]. Some investigations of human and animal hydrocephalus have failed to identify histological abnormalities in cortical gray matter [47;126;152;154]. However, subtle neuronal pathology such as pyknosis and degeneration has been observed in human cortex [55]. Animal studies have demonstrated decreased synaptic density [83] and reduced complexity of the neuronal dendritic trees [87]. Chronic damage to the cerebral cortex results in reduction in size and number of the large pyramidal neurons [164] and rare neurofibrillary tangles in neurons of the cortex and hippocampus [9;10;20;36;51]. The adverse effect of hydrocephalus on the developing brain structure is supported by documentation of polygyria [96] and disturbed

cellular proliferation and migration [112]. These are apparent in hydrocephalic children with aberrant cerebral function [18].

White matter changes include significant losses of myelin and axons [33;58;127;147]. Axons in periventricular white matter are subjected to compression and stretching that result in the thinning of the corpus callosum and central degeneration [38;54;58;77;105;106;127;152;154;155]. Secondary loss of myelin in the periventricular area has been reported histologically [128;163] and biochemically [53;128]. In severe human ventriculomegaly, the presence of myelin basic protein (MBP) in CSF suggests myelin damage [143]. Clinical investigation of hydrocephalic patients before and after shunting demonstrated a significant decrease in MBP levels [89]. Delayed myelination in developing brains has been documented in young rats with kaolin-induced hydrocephalus [39]. Descending degenerative changes have been observed in the corticospinal tract [77;162].

The degree of damage to the ependyma varies from nothing [11] to stretched or torn [21;153] to totally destroyed [129]. The severity of ventriculomegaly and the rate of expansion of the ventricles determine the extent of destruction. The normally cuboidal or columnar ependymal cells will flatten, providing an increased surface area [34;116]. If the ventricles enlarge very slowly, the ependymal lining may remain intact [24]. Damaged ependyma is minimal over the caudate nucleus and more

severe over white matter along the roof and dorsolateral angles of the lateral ventricles [115;124]. This vulnerability of certain locations correlates with the degree of stretching encountered by the underlying tissues. Whether ependymal loss is biologically significant remains unclear.

1.5 Mechanisms of damage

Hydrocephalus research is encumbered by the multifactorial nature of this disorder. The multiple pathological mechanisms operating in concert as the brain undergoes compression and distortion require temporally designed studies. Timing of a given process can help differentiate primary versus secondary pathophysiologic mechanisms associated with hydrocephalus. In general, primary mechanisms occur in the early stages of hydrocephalus. McAllister and coworkers summarized the primary pathophysiologic mechanisms as compression, stretch, interstitial edema, blood-brain barrier breakdown and intraventricular hemorrhage [97], the first two being the most apparent. Compression may result in thinning of the cortical mantle to less than 2 cm in a human brain, with unpredictable neurological deficits [62]. Axonal stretch is probably just as damaging on the cortical mantle as compression. The expandable skulls encountered in pediatric patients allow more traction and less compression. Centrifugal expansion of brain would result in considerable distortion of gray and white matter. The growing

axonal projections might have difficulty finding normal pathways in such an environment.

Experimental and clinical studies have demonstrated reduced cerebral blood flow (CBF) during hydrocephalus [29;56;57;64;70;80;136;150]. Whether the reduction in CBF reaches an ischemic level (18 ml/min per 100g) [67] is still controversial. However, this threshold may not be applicable to chronic conditions such as hydrocephalus. Studies correlating CBF and metabolism in hydrocephalus showed evidence of altered energy utilization from aerobic to anaerobic glycolysis [94;142] and accumulation of lactic acid using MR-spectroscopic imaging [19]. Our own data [92] suggests that alteration in perfusion in the white matter may be present as early as the first day after induction of hydrocephalus. Hypoperfusion has been demonstrated in white matter of hydrocephalic animal brain by various methods [19;29;44;70]. This supports hypoperfusion, if not ischemia, as an important primary pathophysiological mechanism involved in hydrocephalus.

Hakim and coworkers postulated that brain compression occurred at the expense of venous blood and extracellular water [61]. This is supported by observations of decreased water content in the cortex of hydrocephalic rabbits [32], CT scan density changes in humans with hydrocephalus [118], and ultrastructural compression of tissue in hydrocephalic mice [100]. Accumulation of potentially toxic metabolites in brains of hydrocephalic rats and rabbits [35;40;164] leads us to

hypothesize that extracellular fluid flow or molecular diffusion through the extracellular compartment is impaired following hydrocephalic compression of the brain. Our MR data in adult rats suggest compression of both cortex and striatum [92]. An increase in tissue water is supported by increased T2 signals shown in several studies [4;12;157] including our own data [92]. This edema was noted at eight days following induction of hydrocephalus.

1.6 Recovery post-shunting

The improvement of functional outcome following shunting is well documented in human patients and has been demonstrated [37] using the rat model. Hydrocephalus was induced using kaolin injection into the cisterna magna of three-week-old rats. Shunting after one week largely averted learning disabilities. Delay in myelination in the cerebral white matter of immature rats was also reversible using diversionary shunting of CSF [39]. A significant difference in myelin-related enzyme activity in the corpus callosum was observed when comparing the early (one week) versus late (four weeks) groups of shunted rats. Miyazawa studied both behavioral and histological outcome of shunting H-Tx rats with congenital hydrocephalus. A relationship between learning disability and impairment of synaptogenesis was observed. Quantitative Golgi studies of the cortex and neurobehavioral evaluation with light-dark discrimination tests using a

Y-maze were done [107]. Shunting of these H-Tx rats at four weeks of age restored the laminar arrangement of the cortex, but the density of dendritic spines did not recover. Shunting, if performed early enough, can result in brain recovery, specifically myelination and synaptogenesis [33].

1.7 Role of neuroprotection in hydrocephalus

Potentially, numerous neuroprotective strategies for treatment of central nervous system (CNS) injury can be applied. For the purpose of this thesis, I will focus on the role of altered Ca^{2+} homeostasis as a possible mechanism of brain injury. Many different pathophysiological aspects of damage include influx of calcium as a final common pathway of cell death. Cellular energy failure following trauma or ischemia can disrupt many functions, resulting in the breakdown of the membrane potential and influx of calcium. Acidosis, increased anaerobic glycolysis, decreased energy-dependent ion extrusion and decreased cytoskeletal assembly contribute to the demise of membrane potentials [148]. Disruption of membrane potential will reverse the Na^{+}/Ca^{2+} exchange, open voltage sensitive calcium channels (VSCC), contribute to glutamate excess and therefore up-regulation of glutamate receptors activity [131]. Other sources of Ca^{2+}, which contribute to the influx, are internal stores and mitochondria [148]. High

concentrations of intracellular calcium can result in up-regulation of certain enzymes. Calpains, which are calcium-dependent neutral cysteine proteases [102], once activated will cause degradation of the cytoskeletal proteins such as spectrin, tau, tubulin, MAP-1, MAP-2 and ankyrin [22;26;78;88;7;74]. In the brain, the role of calcium for axonal degeneration [132] and neural tissue damage is supported by several in vitro and in vivo studies [8;43;110;138;139;149]. Strategies for the treatment of calcium neurotoxicity can target any of the above mechanisms. Recently obtained unpublished data (Del Bigio et al, 2000) indicates that both Ca^{2+} and calpain are increased in white matter of hydrocephalic rats and that calpain may be activated.

The up-regulation of L-type calcium channels has been demonstrated using immunostaining of white matter with ischemia experiments[156]. The L-type channel is one of several voltage gated calcium channels, which are found in vascular smooth muscle glia and neurons[15]. Nimodipine, a blocker of dihydropyridine-sensitive (L-type) calcium channels, has been in routine clinical use since the early 1980s. The presumed mechanism of action is the reduction of Ca^{2+} influx by blocking L-type Ca^{2+} channels. This resulting decrease in Ca^{2+} influx may provide cytological protection. This lipophilic blocker, nimodipine was noted for its greater brain distribution and direct neuronal action in the rat [133]. Another explanation may be related to blood flow. Langley and Sorkin reviewed the pharmacodynamic and pharmacokinetic properties of nimodipine[85] and

increases in both the cerebral blood flow and diameter of cerebral arterioles were shown in animals and humans. Neuroprotection could therefore be the result of the prevention of ischemia.

Clinical studies have demonstrated the benefit of nimodipine in subarachnoid hemorrhage patients [3;101;119]. Nimodipine has also been used in pediatric patients with migraines[91]. Nimodipine has few side effects and is well tolerated by patients. One of the side effects, hypotension, has been used in the clinical management of hypertension [151]. Nimodipine has been used in clinical scenarios such as head injury [63] and other experimental work [161]. However, the use of nimodipine as a neuroprotectant in hydrocephalic patients has not been reported.

1.8 Promotion of regeneration

The CNS reaction to trauma includes sprouting of neurites from central axons [122]. Up-regulation of growth associated protein (GAP-43, also called as B50, F1, pp46 and neuromodulin) has been documented in the periventricular region of hydrocephalic rats [164]. This membrane-linked protein is involved in transduction of intra and extracellular signals that regulate the cytoskeleton of the nerve ending [16] and is associated with axonal elongation and neurite sprouting [17].

Impediments to regeneration include obstructing reactive glial cells, inhibitory products from myelin breakdown [114], and the absence of appropriate growth factors [114]. Central administration of exogenous neurotrophic factors has been shown to prevent loss and promote recovery of damaged central neurons and axons [76;125]. Cuello demonstrated enhanced synaptogenesis with neurotrophic factors in cerebral cortex of adult animals [27]. Rats with traumatic brain injuries treated with nerve growth factor (NGF) exhibited a superior cognitive recovery compared to control rats [140]. The subependymal zone is potentially an important site for generating new oligodendrocytes from stem cells [25;46]. Mitogens such as platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), and insulin-like growth factor (IGF-1) stimulate the proliferation of oligodendrocyte precursors that reside in the white matter [46;48;60].

One of the challenges in administering neurotrophic factors is the difficulty penetrating the blood brain barrier [158]. Strategies to overcome this obstacle include the use of gene manipulation to allow cellular delivery of ciliary neurotrophic factor (CNTF). This method has demonstrated some success in minimizing motor and cognitive dysfunction in animals with striatal lesions [49]. Intraventricular delivery of NGF[59] has been used in patients with Alzheimer's disease [50].

CNTF has been noted to protect central neurons following cerebral contusion or infarction [84] and to prevent motoneuron death after axotomy [23;134;144]. Axon regeneration in vitro has been demonstrated with CNTF [145] and CNTF is believed to play a role in survival of oligodendrocytes [160]. CNTF has cytokine-like side effects because of its similarity to interleukin-6 [69], which can result in significant weight loss. AXOKINE ®, a genetically engineered second-generation form of CNTF, which has fewer cytokine-like side effects and greater potency, has been developed as a possible therapeutic agent [86]. It protects striatal neurons in kainate-induced animal models of Huntington disease [6] and photoreceptors [86]. Based on this information, the choice of CNTF delivered into the ventricles of hydrocephalic rats seemed to warrant investigation.

1.9 Summary

Hydrocephalus is second only to spina bifida as the most frequent congenital neurologic malformation in North America [159]. In spite of the advances in technology, the surgical management of this disease is still plagued with a high complication rate [45]. This surgical complication rate incites us to improve upon the management of hydrocephalus and forces us to explore other therapies. The role of adjunctive drug therapy is the focus of this study. Neuroprotection prior to shunting and enhancement of neuronal recovery after shunting will be investigated with nimodipine and AXOKINE ® respectively in an animal model of hydrocephalus.

1.10 Hypotheses

We hypothesize that:

1- Periventricular axon integrity is damaged by calcium influx in hydrocephalic rat brain and administration of a calcium channel blocker, nimodipine, to young hydrocephalic rats will minimize brain damage and improve functional outcome.

2- Administration of an exogenous neurotrophic factor, AXOKINE ®, by intraventricular delivery following shunting of young rats with experimental hydrocephalus will result in improved recovery and better functional outcome.

2.0 Materials and Methods

All animals were treated in accordance with the guidelines set forth by the Canadian Council on Animal Care. The institutional animal ethics committee approved all protocols. A total of 144 male, three-week-old, Sprague-Dawley rats with an average initial weight of 50g were used for the experiments. The animals were supplied locally by Charles River Valley breeding company and had free access to food and water at all times.

Nimodipine was purchased from Research Biochemical Incorporated (Natick, MA). AXOKINE ® was donated by Regeneron Pharmaceuticals (Terrytown, NY). ALZA mini-osmotic pumps (model 2002) were purchased from ALZA Corporation (Palo Alto, CA). The antibiotic used was cefazolin sodium, purchased from Novopharm (Toronto, ON). The tubing used for intrathecal delivery of AXOKINE ® and as shunts was Micro-Renathane (0.040 o.d. x 0.025 i.d.) from Braintree Scientific Inc. (Braintree, MA).

2.1 Induction of hydrocephalus

Rats were anesthetized using ketamine/xylazine (90/10 mg/kg i.m.). After shaving and washing the skin over the nuchal area of each rat, mild flexion of the

neck provided better localization of the foramen magnum. A 27 gauge needle was inserted into the cisterna magna then sterile kaolin suspension (25 % in 0.9 % NaCl W/V) was injected slowly, delivering a total volume of 0.05 ml. Control animals received normal saline injection of the same amount.

2.2 Magnetic resonance imaging

Rats were anesthetized using ketamine/xylazine (90/10 mg/kg i.m.). Rat brains were visualized by magnetic resonance imaging in a 7 Tesla Bruker Biospec/3 7T/ 21 cm horizontal bore spectrometer (Karlsruhe, Germany) with a radiofrequency coil surrounding the head. Coronal slices, 1.0 mm in thickness, TR of 3.0 s, single echo TE 80 msec, were used to obtain T2-weighted images; eight to ten contiguous coronal slices were obtained. Lateral ventricles size was assessed on the coronal images anterior to the third ventricle. Ventricle size was expressed as a ratio determined by dividing the width of the frontal horns of the lateral ventricles by the width of the cerebrum. Examples of different degrees of ventriculomegaly can be seen in Figure 1.

2.3 Nimodipine treatment

Two pilot experiments were conducted. These provided information which was used to design subsequent experiments. The difficulties encountered with the protocols will be elaborated in the Discussion section of the thesis.

The first group of 30 rats was divided into six arms based on drug, dose and kaolin or sham injection. A high (20 mg/kg/dose) or low (2.0 mg/kg/dose) dose of nimodipine was administered subcutaneously (s.c.) in a 20% dimethylsulfoxide (DMSO) and 5% ethanol vehicle solution. The control consisted of the solution only. The twice a day (BID) administration of the drugs was initiated one day after the injections (kaolin or sham) and continued for two weeks. All rats were weighed weekly and subjected to the same serial behavioral testing (see below). After four weeks the animals were imaged and euthanized within 24 hours. This group will be referred to as the nimodipine pilot 1.

The second pilot group of 34 rats was divided into four arms consisting of a low nimodipine dose (2.0 mg/kg/dose s.c.), a high nimodipine dose (10 mg/kg/dose s.c.), an oral (PO) nimodipine dose (10 mg/kg/dose), and control. Frequency of drug administration was unchanged, BID. A new vehicle was introduced in order to facilitate mixing nimodipine into a solution that would be administered subcutaneously and orally. The vehicle was initially 10% PEG and

5% ethanol, but due to renal acidosis, the vehicle was changed after four days of treatment. The new solution consisted of 20% dimethylsulfoxide (DMSO) and 10% ethanol; the nimodipine dose remained unchanged. These animals were left untreated for two weeks after the kaolin injection, treated for two weeks, imaged and euthanized within 24 hours. Total duration of the experiment was four weeks. The results of this group will be referred to as Nimodipine pilot 2.

The third group, this group will be referred to as the experimental group, saw 40 rats divided into two arms, control and nimodipine (1.8mg/day s.c.). The vehicle solution was 50% DMSO and 5% ethanol. These animals were left untreated for two weeks. The first MR image was then obtained and the animals were stratified into two subgroups based on their ventricular size – large or moderate. Both groups, large and moderate, were then randomized to receive either control vehicle or nimodipine. Four rats with very small or normal ventricles were excluded, leaving 18 rats in the control and experimental arms. At the time of surgery, all animals received a mini-osmotic pump (Alzet, model 2002). The pumps were inserted subcutaneously using aseptic technique while the animal was anesthetized with ketamine/xylazine (90/10 mg/kg, i.m.). The mini-osmotic pumps contained 243.1 +/- 4.53 microliters and a mean pumping rate of 0.55 +/- 0.02 microliters/hour. The skin incision, located at the base of the skull, measured 2 cm in length and was closed using staples. The second MR image was

obtained prior to euthanization of all animals five weeks after the kaolin injection. The animals were subjected to five weeks of behavioral testing, one prior to kaolin injection, two without treatment and two more with treatment. The rationale for this duration is based on several facts. The mortality rate of rats with hydrocephalus becomes significant after four weeks [39]. The recommended time allowed for the implanted pumps is two weeks. The results of this group will be reported as Nimodipine experiment.

2.4 AXOKINE ® treatment

Thirty-six rats were divided into three experimental arms. Two of these received AXOKINE ® by intraventricular route at a dose of 1.4 μg/day or 14.3 μg/day and the third received only the vehicle (phosphate buffered saline, PBS). Behavior testing was started one week prior to the kaolin injection and continued for six weeks after. The animals were left untreated for three weeks following the induction of hydrocephalus. The first MR images were acquired at this point and then rats were divided into a large and moderate ventricle size ratio. Each group was then randomized to receive an ALZA mini-osmotic pump (model 2002) filled with one of three solutions: control, low dose AXOKINE ® or high dose AXOKINE ®. The pumps had been primed for 24 hours, as set forth by ALZET

protocol. The rats were shunted and an intrathecal pump implanted under anesthesia induced by ketamine/xylazine (90/10 mg/kg). All implanted material was gas sterilized with ethylene oxide prior to insertion.

The rats were placed lying flat with the head secured to a platform. After shaving the scalp and washing the skin with alcohol and povidone solutions, drapes were applied. This aseptic technique was used for all rats and maintained for the entire surgery. A midline scalp incision measuring approximately 3 cm was made. The skull was perforated (0.5 mm) bilaterally, 2 mm from the midline and 4 mm anterior to the transverse sinus, using a power drill with sterile drill bits. These perforations allowed insertion of the shunt and of the distal end of the tubing, which was connected to the mini-osmotic pump. Micro-Renathane tubing (0.04 mm in o.d.) was used for both the shunt and pump tubing. The shunt tubing was inserted into the right lateral ventricle and the pump tubing into the left. The insertion was performed under magnification to ensure minimal trauma. The depth of insertion was approximately 2 mm. Micro-Renathane tubing had a sterile silicone rubber stopper glued with cyanoacrylate glue 4 mm from the distal end in order to facilitate anchoring. Anchoring was achieved by placing a suture into the temporalis muscle and ligating the tube proximal to the silicone in order to prevent slipping out of the ventricles (Figure 2). The scalp incision was closed using staples. The animals were left to recover for several days prior to resuming

behavior testing. Cefazolin sodium was administered intramuscularly fifteen minutes before surgery and one-day post, at a dose of 25 mg per kilogram.

The second MR images were acquired after three weeks of treatment and prior to euthanization of all animals.

2.5 Behavioral assessment

An objective battery of tests to assess motor function and cognition requires accuracy and reproducibility. By quantitatively evaluating certain parameters, the rate of improvement can be assessed and compared between groups. More subtle differences require a greater number of points of comparison in order to achieve significance.

Motor function was evaluated using a chamber with infrared beam sensors located every 2.65 cm to detect movement (Opto-varimex, Columbus Instruments). After one minute of familiarization, movement is recorded automatically in both horizontal and vertical planes for 15 minutes. The vertical count is triggered when the animal interrupts the infrared beam located 15 cm above the monitored surface. The vertical count is referred to as the exploratory movement. The ambulation count is generated when the animal sequentially breaks more than two adjacent infrared beams in the horizontal plane. A third count, non-ambulatory movement, is generated from any repeated interruption of a single infrared beam; this count could represent activities such as scratching or grooming. The total count is the sum of ambulatory and non-ambulatory movements in the horizontal plane only.

The ambulatory agility of the rats was quantitatively assessed using a rotating drum (Economex, Columbus Instruments). Two trials were performed. The first was to assess endurance. This required the rat to stay on the drum (10 cm diameter), which was rotating at a constant speed of 5 rpm, for a maximum of two minutes. In the second trial, the initial speed was set at 2.5 rpm and accelerated at a rate of 0.1 rpm every second. The time recorded represents the moment the rats were placed on the rotating drum until they fell off.

Recording the time required by the rat to swim 1.5 meters also assessed motor function. A pool was constructed, measuring 20 cm by 40 cm with a length of 150 cm, and filled with water at room temperature. Rats were placed at one end and time was started immediately. Rats experiencing difficulty with the task or requiring more than 60 seconds to complete the task were removed and rested for 60 seconds.

Learning and memory function were evaluated using a modified Morris water maze [141]. Validation of performing three trials in one day to assess the animals' ability to learn was done by Kraemer and coworkers [82]. Testing was done in a dimly lit room with a pool (90 cm^2, 25 cm deep) filled with 22°C water. A platform (13 cm round) was placed 1 cm below the water surface. The water was made opaque by adding kaolin. A single wall was illuminated to provide directional cues. The rats were placed in the center of the pool and allowed to

swim until they found the platform. A trial consisted of four attempts to find the platform. The rats faced a different direction with each attempt. If the rat failed to complete the task in 60 seconds it was given a 60 second rest before the next attempt. Three trials were performed during the course of the day; each separated by a two-hour interval. Data generated consisted of the averages of the four attempts in one trial. If the rats were capable of learning, the mean swimming time is expected to decrease progressively during the course of the day. If the rats improved their motor ability or retained memory of the task, each day's initial trial time should also decrease from week to week.

2.6 Histological and immunohistochemical assessment

Rats were sacrificed by an overdose of pentobarbital. The vascular system was cleared by perfusion with ice cold 0.1 M phosphate-buffered solution (PBS) and their brains were removed immediately. Brains were then cut coronally at the level of the optic chiasm. Samples from the corpus callosum, hippocampus, cerebellum, parietal and frontal lobes were dissected and frozen in liquid nitrogen, then stored at –80°C (Figure 3).

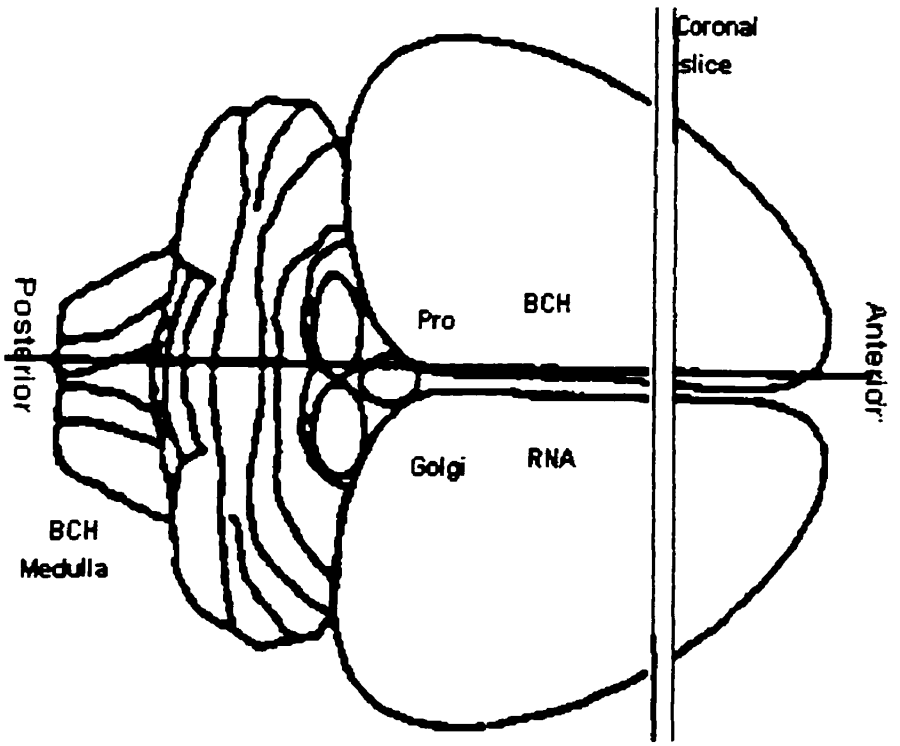

Figure 3: Dorsal view of a rat cerebrum including the cerebellum and medulla, the longitudinal axis is going from right to left. Location of cortical sampling for the enzymes (PRO), biochemical (BCH), Golgi, and Ribonucleic acid (RNA) studies are labeled. The coronal cut is at the level of the optic chiasm.

The remaining pieces of rat brain were immersion fixed in 10% buffered formalin. The anterior portions of the frontal lobe of the brain, cut coronally at the level of the optic chiasm, were sectioned and stained with hematoxylin and eosin (H&E), as well as solochrome cyanine (S/C) for visualization of myelin. Immunohistochemical methods were used to detect myelin basic protein (rabbit polyclonal anti-MBP, 1/300 dilution; Dako) on paraffin sections at the coronal level of the anterior commissure. This antibody was revealed using biotinylated secondary antibodies (1/300 dilution; Jackson) followed by streptavidin-HRP and diaminobenzidine. This method was used to define and measure the thickness of the corpus callosum (CC) at two different points between the midline and the most lateral part of the CC anterior to the fimbria (Figure 12). Immunohistochemical staining using rabbit polyclonal anti-GFAP (Dako; 1/100 dilution) was used to assess reactive astrogliosis and anti-neurofilament 200 kD (Biogenex; 1/200 dilution) was used to assess periventricular axons. The intensity of GFAP immunoreactivity (Figure 13) was assessed on the slides using the spot metering system of a Nikon Microphot FX epifluorescence microscope as an optical densitometer. At 20X objective magnification, the spot meter registers a circular area 50 μm in diameter. With consistent illumination and filter settings for transmitted light, the photometer reading was an effective measure of labeling intensity. All brain sections were sampled at four different points along the corpus

callosum, two areas in the fimbria and four different areas in the gray matter. These readings were then averaged and subtracted from background optical densitometry readings. This method has been previously validated by Del Bigio et al [36].

2.8 Statistical assessment

All results are reported as a mean with the corresponding standard error (+/-) in table or graph format. All data was tested for normal distribution. The p-values for the nimodipine experiment were generated using t-test and the AXOKINE ® experiment required ANOVA.

3.0 Results

3.1 Nimodipine experiment

The results of the nimodipine pilot 1 will not be reported in detail due to the lack of significant ventriculomegaly. The rats had a ventricular size index of 0.48 +/- 0.03. This did not sufficiently affect their functional status. Kaolin injections were adequate based on gross and microscopic observations of kaolin in sufficient amount and appropriate location. The most plausible explanation for this failure is the vehicle used and its timing in relation to the kaolin injection. Dimethylsulfoxide, DMSO, is believed to have anti-inflammatory properties. In vitro studies have shown inhibition of platelet aggregation [130] and inhibition of microbiocidal activity of neutrophils [14]. Inhibition of neutrophil recruitment mediated by interleukin-8 in airway epithelium has been demonstrated in vivo using dogs [93]. In the Mongolian gerbil, there is some reduction in size of cerebral infarction when treated with DMSO [99]. This anti-inflammatory property of DMSO might therefore blunt the induction of hydrocephalus by interfering with the inflammatory response of kaolin injected into the cisterna magna. The CSF pathways are then left without significant obstruction. Timing is also important; in

this pilot we started DMSO administration the day after kaolin was injected, thereby allowing the DMSO to inhibit inflammation.

In nimodipine pilot 2 we used a different solvent as a vehicle. Polyethylene glycol was used and resulted in the renal acidosis and subsequent death of a quarter of the experimental animals. Attempts to salvage the experiment by treating the acidosis with injections of sodium bicarbonate were met with limited success. The vehicle had to be changed back to DMSO. The subcutaneous injections caused skin necrosis. Due to all of the variables introduced into this pilot and the high mortality rate, the results of this group were excluded. Information from this experiment was used however to guide the dose used in subsequent experiments and to use only the subcutaneous route.

A number of changes in the protocol were introduced in the nimodipine experiment. A two-week period with no treatment was implemented in order to achieve significant ventriculomegaly. The delivery of the nimodipine using Alzet mini-osmotic pumps allowed a more continuous delivery. The dosage of nimodipine was based on the two pilot studies. The previous dose of 20 mg/kg/day seems to have had some effect and was therefore used in this third trial. Other limiting factors include the maximum concentration of nimodipine without precipitation in such a small volume (243 microliters). The insertion of

the osmotic pumps was not a complicated procedure and was tolerated by the animals.

The nimodipine experiment will be reported in detail. The mortality rate for this experiment was 10% (4/40). Two rats died within two weeks of the kaolin injection, the other two within the first month. The control and nimodipine arms were left with 18 animals each. The weight of each animal was recorded three times a week to assess nourishment and development. The weight gain data is presented in Figure 4. The change in ventricular size index from MR images is shown in Figure 5, and as expected both groups had significant progression in comparison to the pre-treatment starting point. However there were no significant differences between the two groups at either time point $p > 0.1$.

Spontaneous activity counts are reported in Table 1, with no significant inter group differences in total, ambulatory or vertical movements. Note that all rats were less active during week 3. This is presumably related to discomfort following the insertion of the Alzet mini-pump.

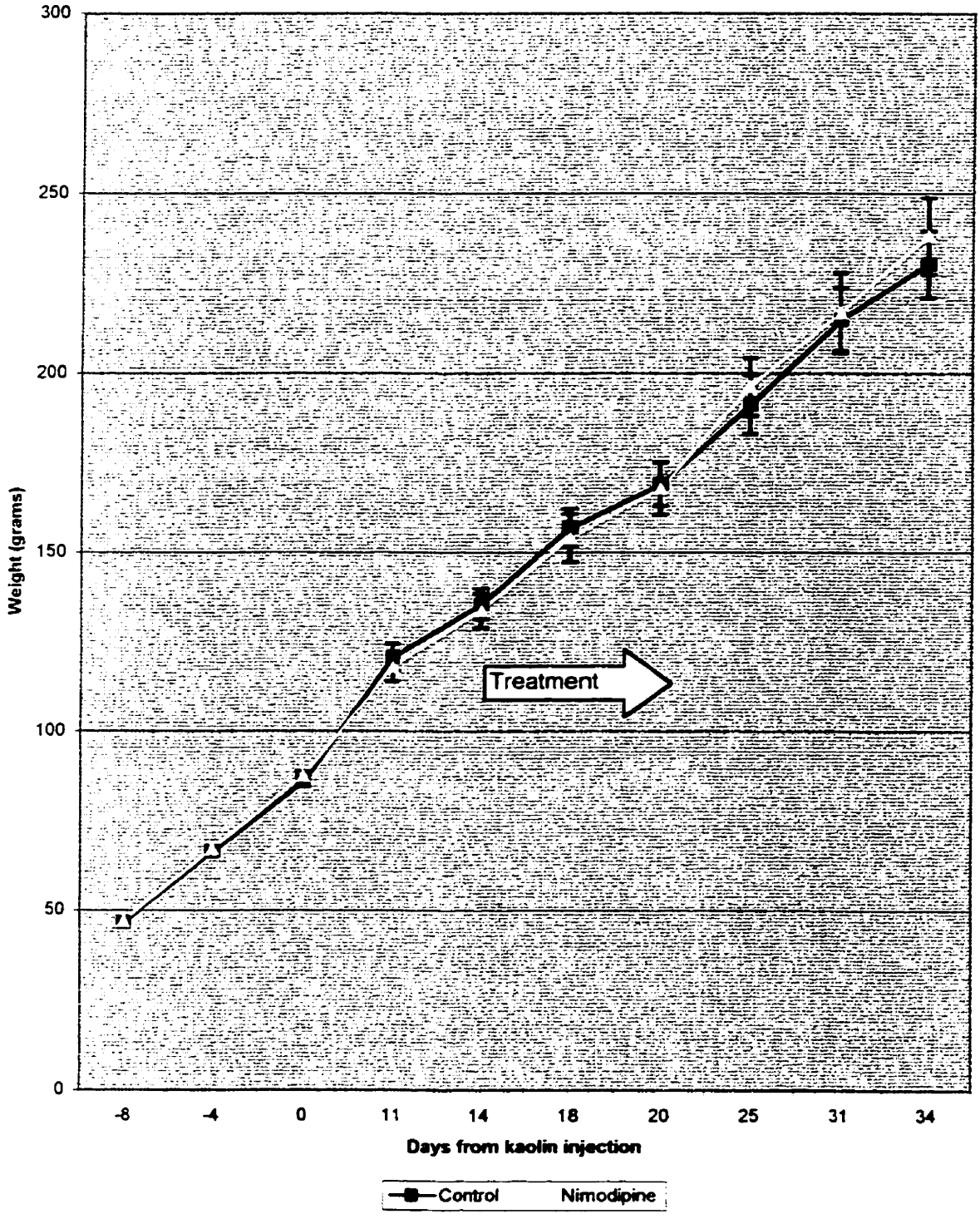

Figure 4: Weight gain of rats following kaolin injection, no significant differences.

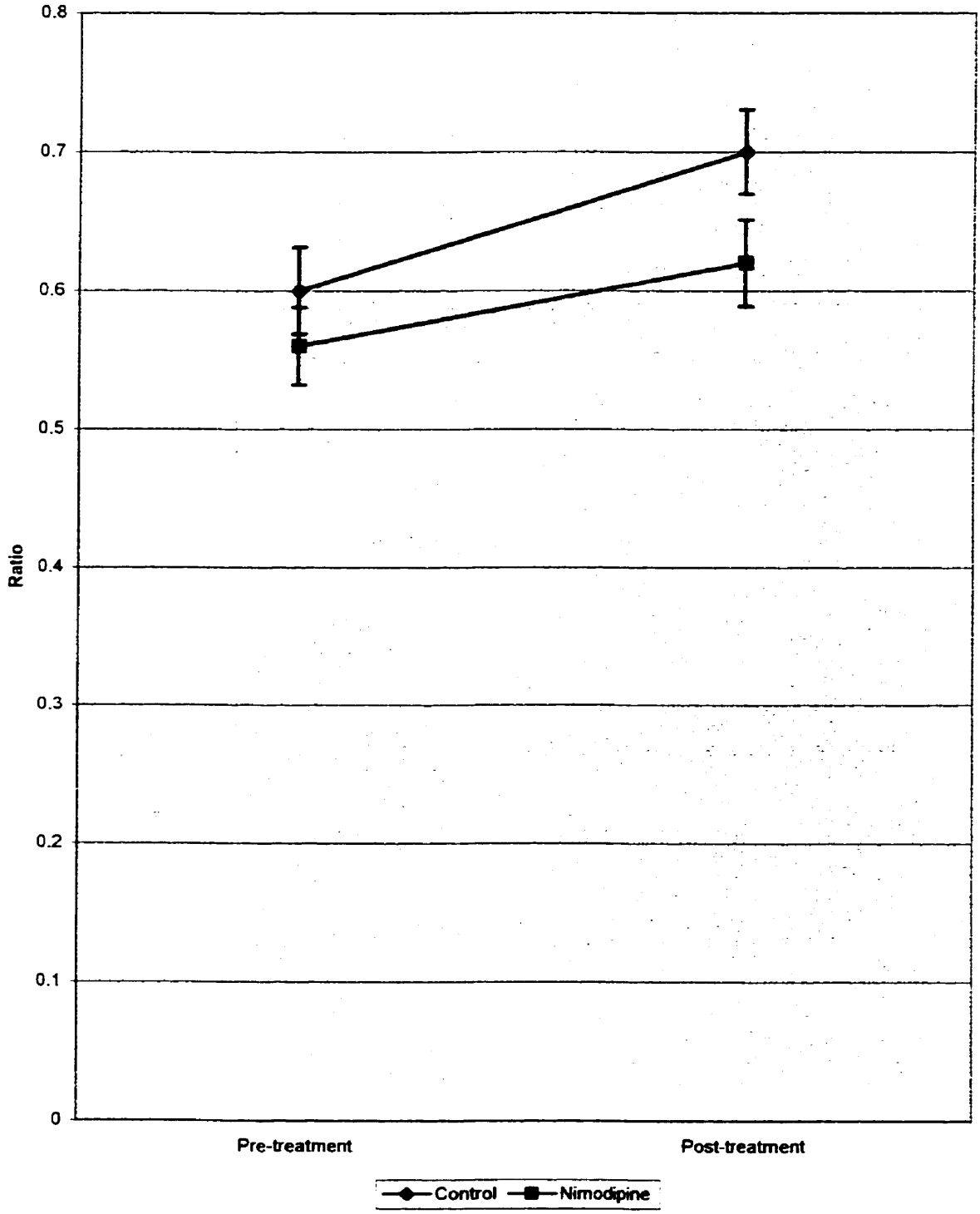

Figure 5: Ventricular size index before and after nimodipine treatment, both increase with $p < 0.004$

Table 1a: Spontaneous activity total count, no significant difference

Time point	Control	Nimodipine
Week 0/ Pre-kaolin	2069.4 +/- 137.3	2061.1 +/- 172.8
Week 1/ no treatment	2266.9 +/- 249.6	2185.0 +/- 575.5
Week 2/ no treatment	2181.4 +/- 252.5	2299.3 +/- 383.4
Week 3/ with treatment	1430.5 +/- 364.0	1213.2 +/- 259.8
Week 4/ with treatment	2157.0 +/- 316.5	1521.1+/- 241.2

Table 1b: Spontaneous activity ambulation count, no significant difference.

Time point	Control	Nimodipine
Week 0/ Pre-kaolin	1217.6 +/- 96.1	1212.1 +/- 130.3
Week 1/ no treatment	1505.9 +/- 207.8	1520.7 +/- 472.9
Week 2/ no treatment	1408.6 +/- 183.0	3126.0 +/- 1686.2
Week 3/ with treatment	926.1 +/- 328.2	794.6 +/- 202.1
Week 4/ with treatment	1508.0 +/- 273.9	1023.6 +/- 201.4

Table 1c: Spontaneous activity vertical count, no significant difference.

Time point	Control	Nimodipine
Week 0/ Pre-kaolin	116.4 +/- 14.8	103.5 +/- 18.2
Week 1/ no treatment	208.4 +/- 40.8	194.7 +/- 44.7
Week 2/ no treatment	328.9 +/- 63.0	314.2 +/- 55.9
Week 3/ with treatment	167.8 +/- 57.5	156.2 +/- 34.9
Week 4/ with treatment	339.3 +/- 49.5	297.2 +/- 58.6

The roller times for constant speed and constant acceleration are displayed in Figures 6 and 7 respectively. Roller times for the rats at constant speed were significantly different in the fourth week, after two weeks of treatment. Roller times at a constant acceleration showed significant differences following treatment in weeks 3 and 4. Both of these results indicate a significantly better performance by the nimodipine-treated rats.

The times needed to swim 1.5 m were significantly different for the nimodipine-treated rats during the treatment period (Figure 8). This supports a superior motor performance by the nimodipine-treated rats. The combined results of the roller times and the swimming times indicate a superior motor performance by the nimodipine-treated rats.

Learning and memory function assessment can be made using the time required by the rats to find the submerged platform. The overall performance of the rats in both groups is depicted in Figure 9. As the rats mature they improve their performance by swimming faster and remembering where the platform is located. This improvement is stunted by hydrocephalus in the control rats. For the first two weeks the times required to find the platform decreased both within the same day and from week to week. The control rats became slower at finding the platform indicated by longer times in weeks 3 and 4. However, nimodipine-treated rats have consistently lower times. The lack of further improvement in

nimodipine-treated rats could be explained by the already short times needed to find the platform. Looking specifically at the first trial of the day, nimodipine-treated rats were significantly faster at finding the platform once treatment began (Figure 10). This indicates retained memory of the task from the previous week. The overall average for the day also improved more significantly with treatment (Figure 11).

The overall behavioral performance of the nimodipine-treated rats was superior, demonstrated by longer times on the roller and shorter times swimming. Although the latter results are influenced by motor performance, they are also suggestive of better learning and memory ability by the nimodipine-treated rats.

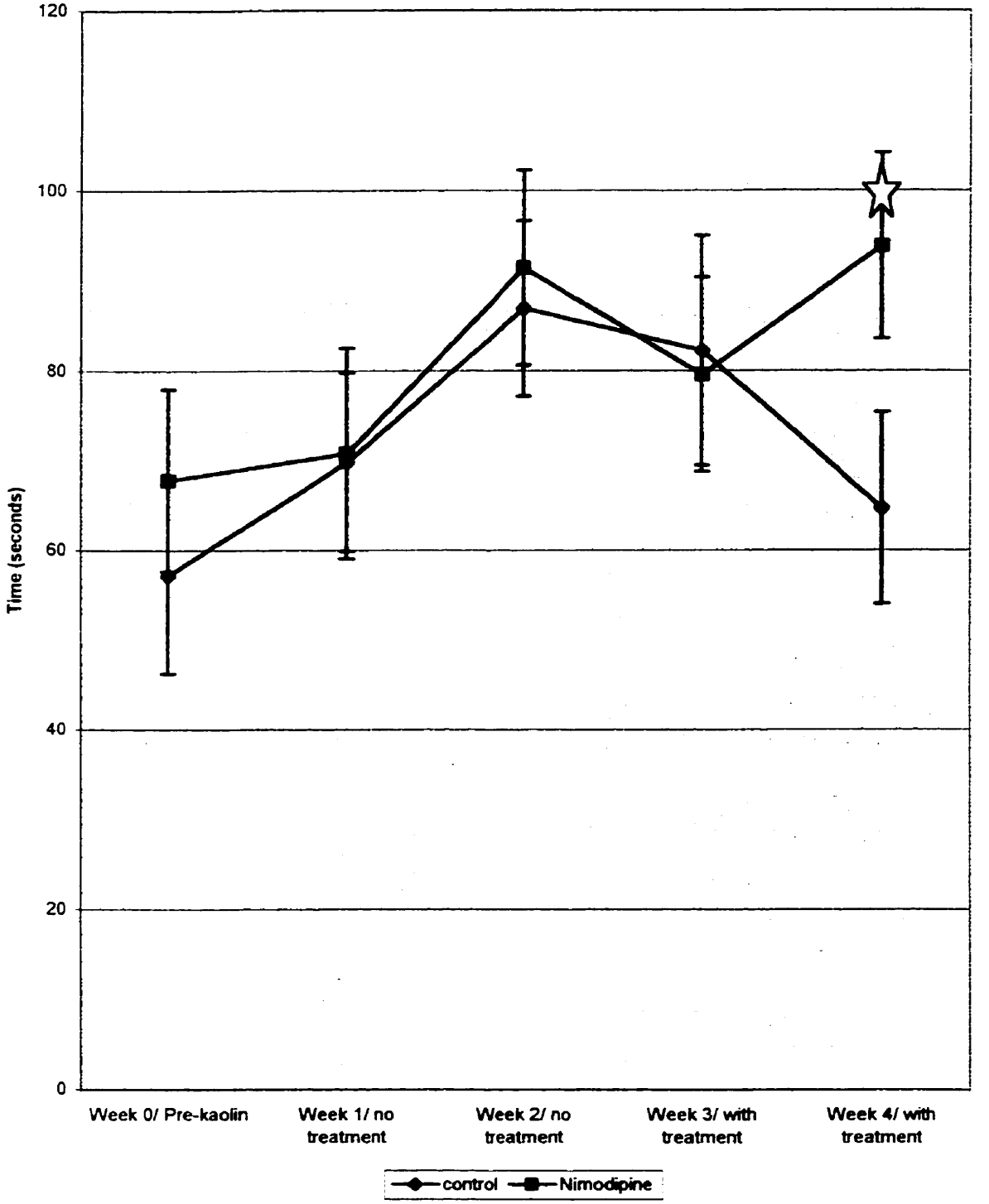

Figure 6: Endurance (time) of rats walking on roller at constant speed (5 rpm) before and after nimodipine treatment, star = $p < 0.001$

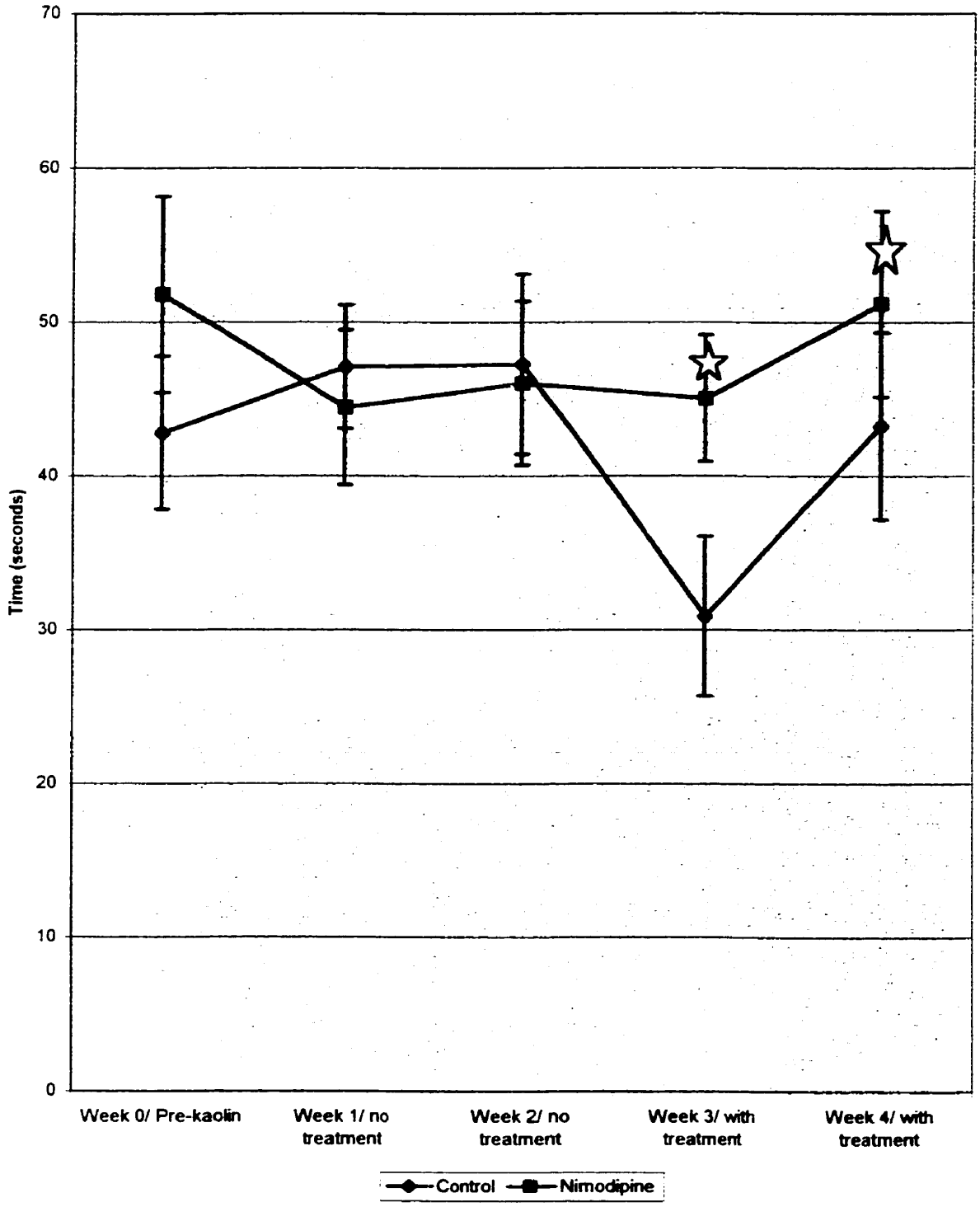

Figure 7: Endurance (time) of rat walking on roller at a constant acceleration (0.1 rpm/second) before and after nimodipine treatment, star = $p < 0.04$

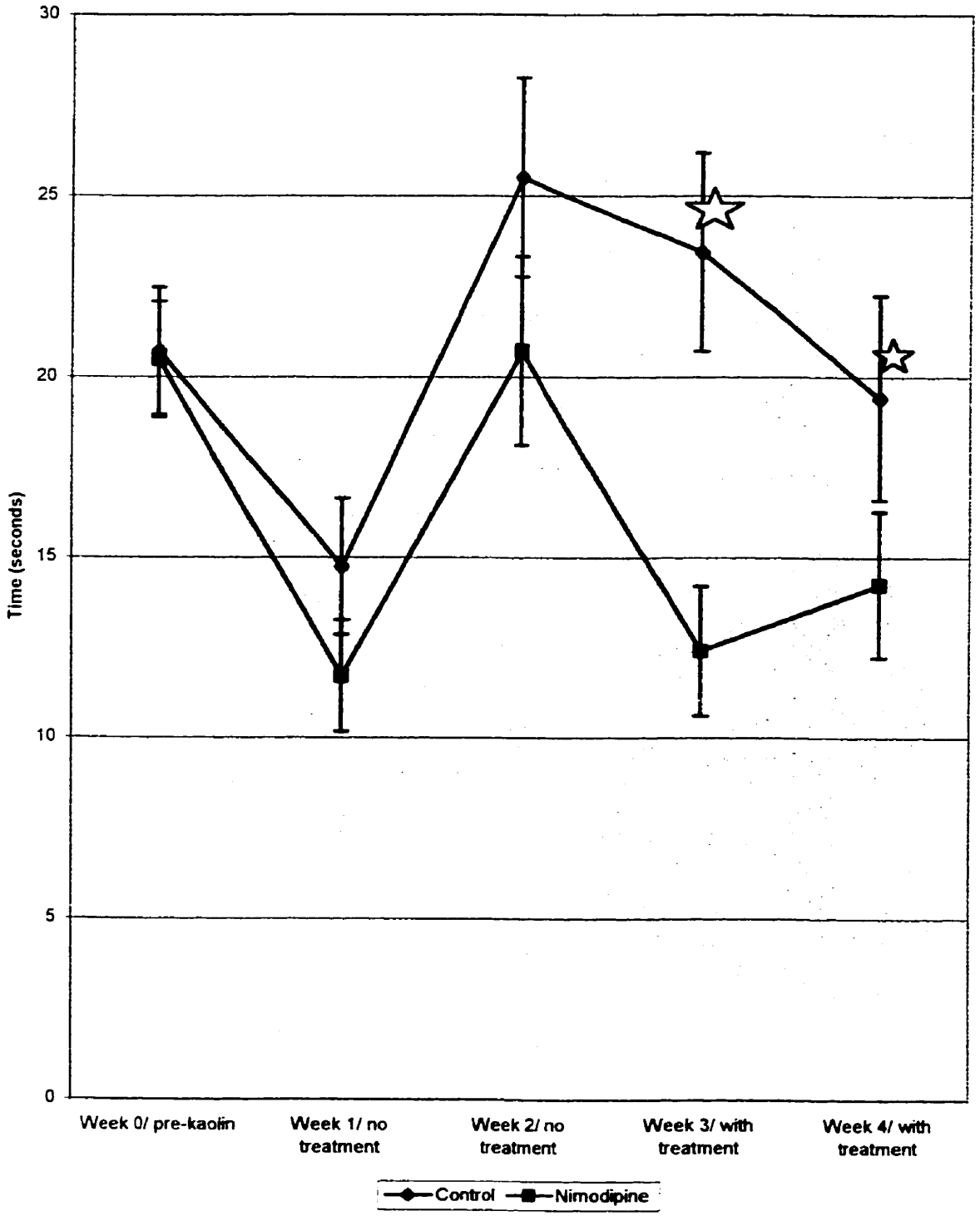

Figure 8: Time required to swim 1.5 m in the nimodipine experiment, star = $p < 0.001$

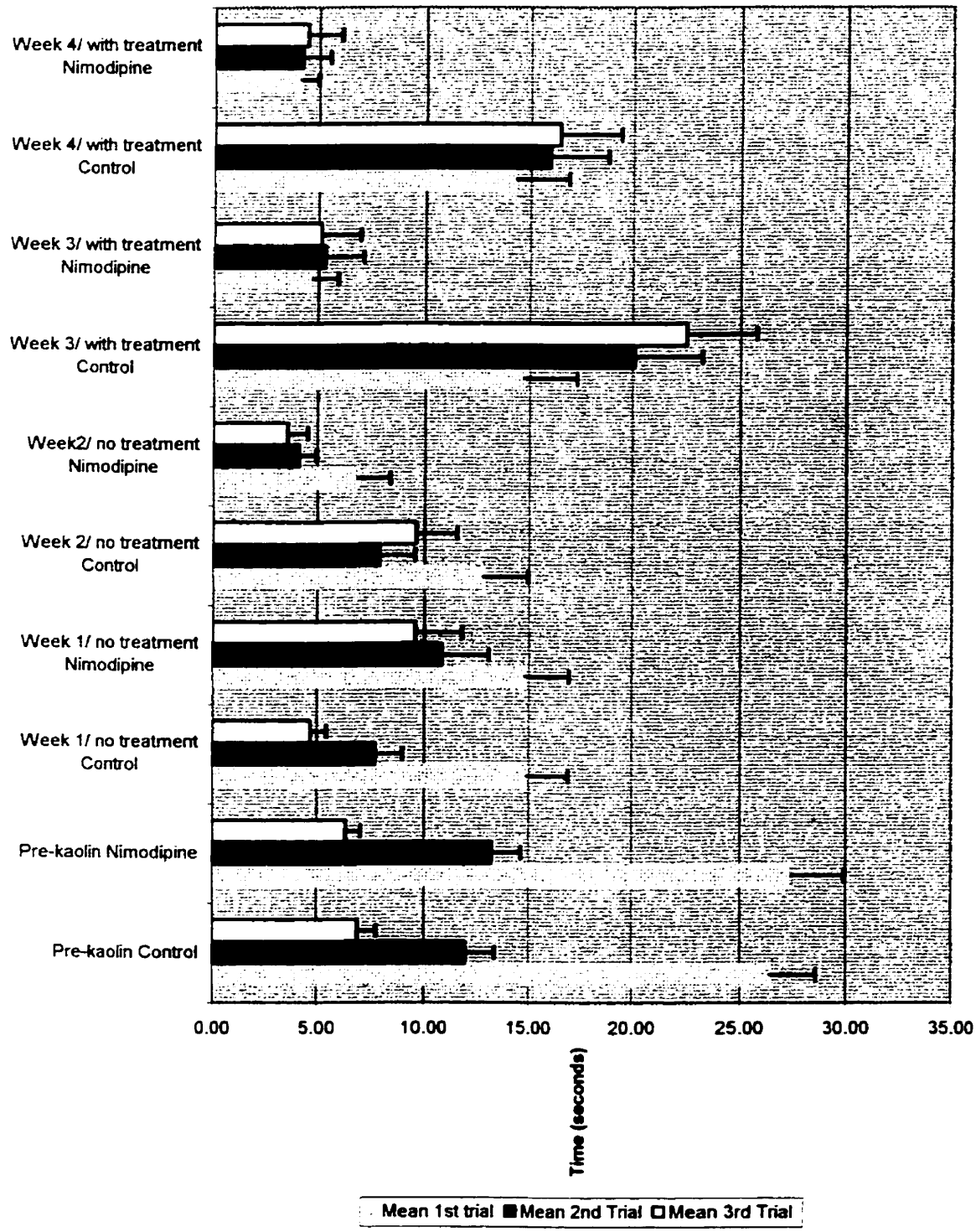

Figure 9: Time required to find submerged platform, each experimental arm is depicted weekly with all three trial of the day.

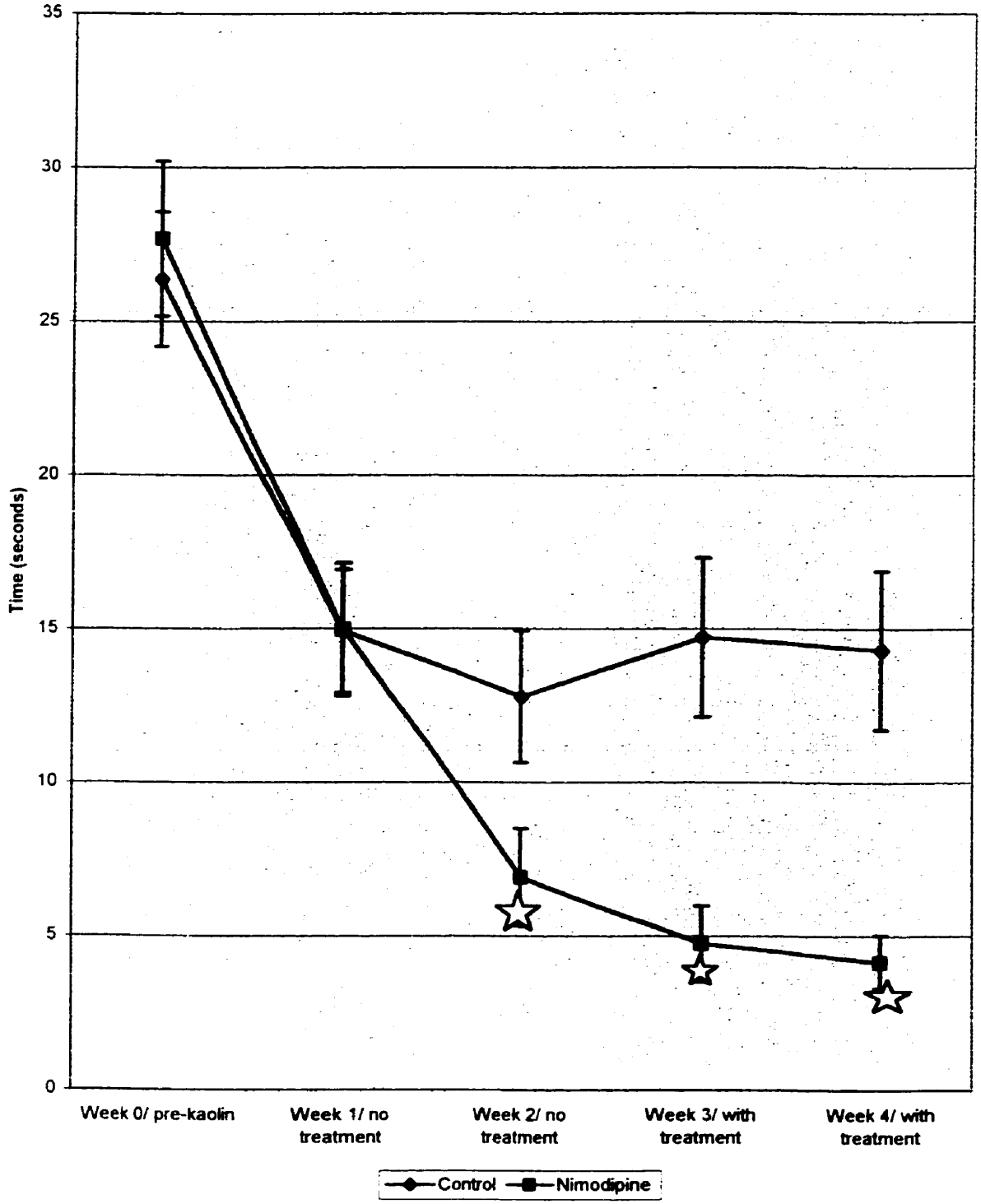

Figure 10: Swimming times for the 1st trial of the day, star = $p < 0.03$

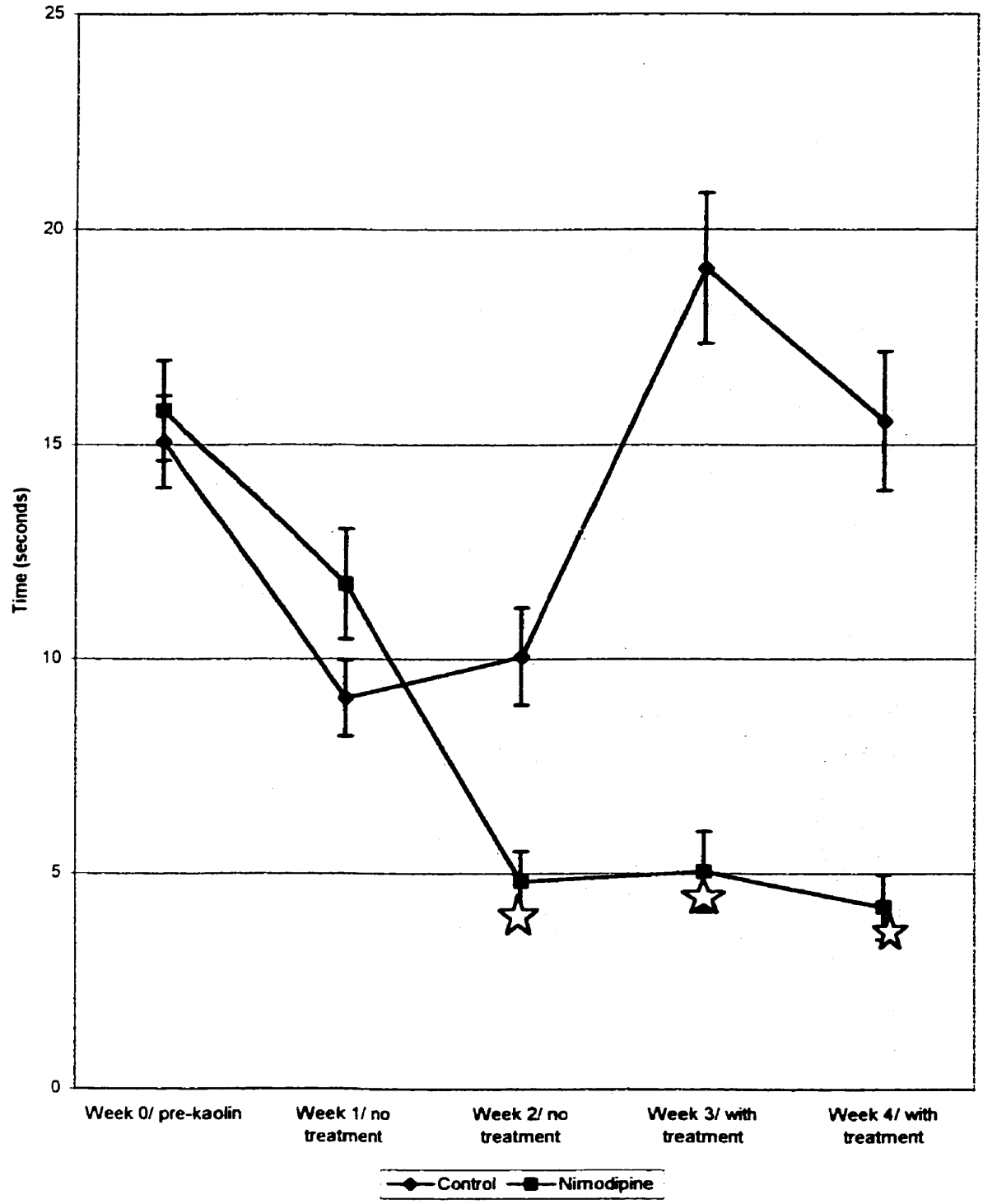

Figure 11: Swimming times for the mean of the three trial in the day, star = $p < 0.005$

Microscopic observations of coronal sections of cerebrum at the level of the optic chiasm were performed. H & E slides clearly showed enlargement of the ventricles and thinning of the corpus callosum. Evidence of ventriculitis or parenchymal lesions was also assessed. Macrophages laden with refractile kaolin particles were noted in the subarachnoid space at the base of the brain and rarely in the ventricles. No obvious differences were noted between the control and nimodipine-treated rats.

MBP immunohistochemical staining was used to assess the corpus callosum (CC) and measure its thickness in a coronal slice at two points. Figure 12 illustrates the corpus callosum of the rat brain at this level. The mean thickness at the midline and lateral aspect of the CC are reported in Table 2. Nimodipine-treated rats had a significantly thicker corpus callosum.

The anti-GFAP labeled slides were used to generate optical densitometric data (Table 3); an example of this immunohistochemical labeling is shown in Figure 13. GFAP immunoreactivity was slightly greater in the nimodipine-treated corpus callosum and fimbria. No significant difference was noted in the cortex for anti-GFAP labeling.

The neurofilament immunohistochemical labeling showing the subtle changes of a swollen axons and varicosities (Figure 14). There were no significant quantitative differences between groups.

Figure12:MBP slide for anatomical detail

Table 2: Corpus callosum thickness measurements, MBP slides, * = p< 0.02

	Midline measurement	Lateral measurement
Control	280.6 +/- 18.6 μm	284.7 +/- 25.8 μm
Nimodipine	343.8 +/- 48.3 * μm	330.0 +/- 31.6 * μm

Table 3: GFAP optical densitometry readings *

Anatomical location	Corpus callosum	Fimbria	Cortex
Control	89.34 +/- 1.82	83.66 +/- 2.87	74.64 +/- 1.33
Nimodipine	92.18 +/- 1.22	87.14 +/- 1.80	75.45 +/- 1.29
P-values	0.02	0.04	0.13

* Method for these readings is on page 28

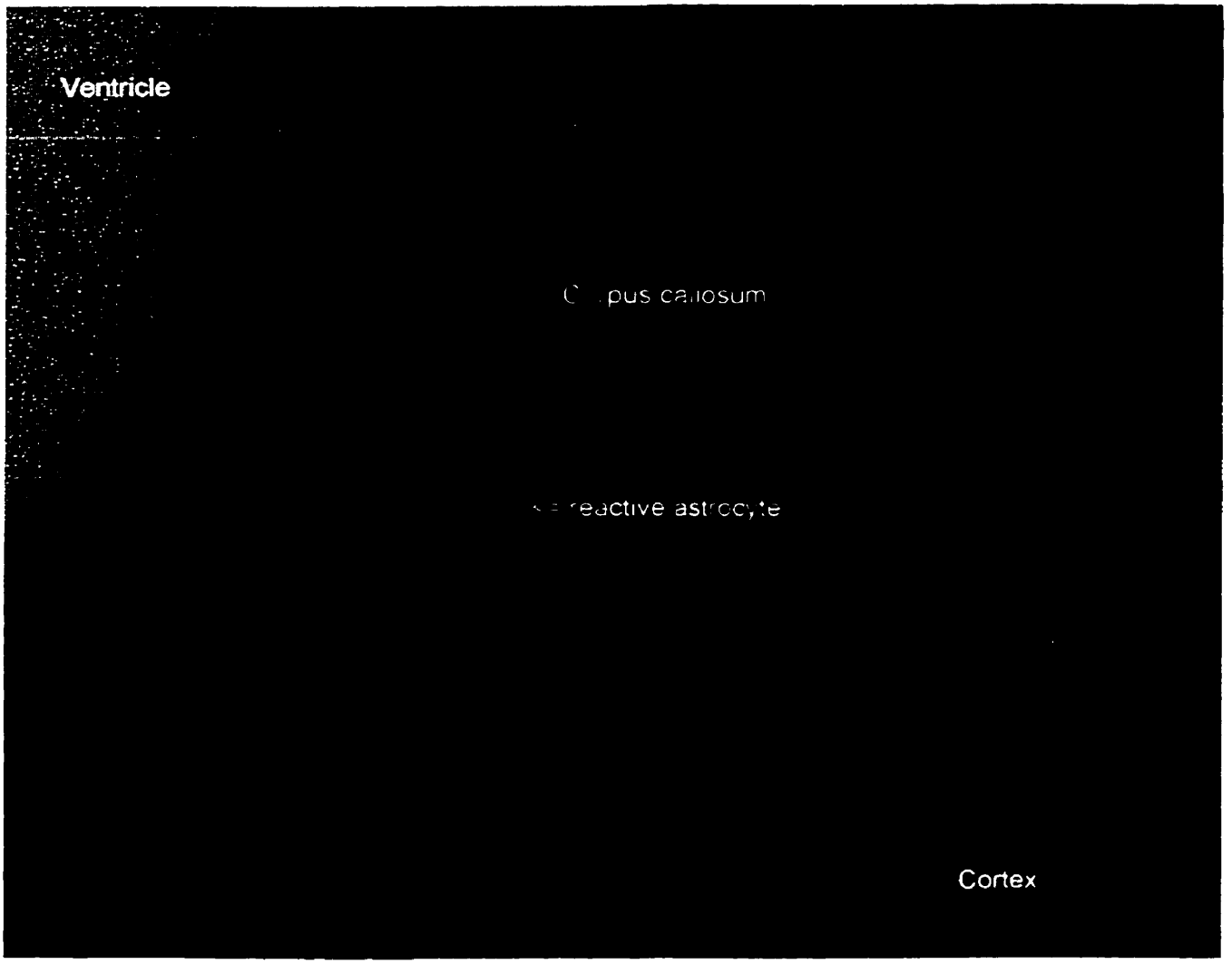

Figure 13: Coronal section of rat brain using anti-GFAP immunohistochemical label showing reactive astrocytes predominantly in the corpus callosum.

Magnification 650X

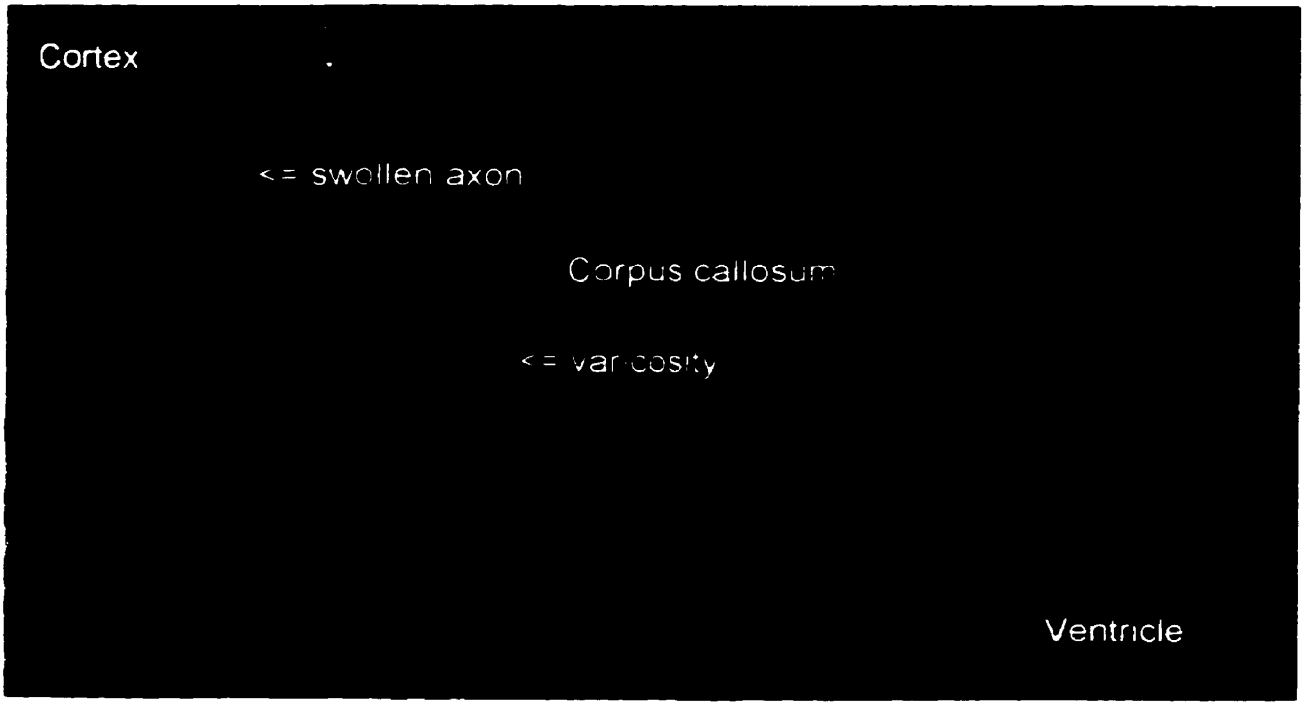

Figure 14: Coronal section of rat brain showing neurofilament immunohistochemical label.
Magnification 700X

3.2 AXOKINE ® experiment

Of the 36 kaolin injected rats used for this experiment, only 18 were used for final analysis. Two died in the peri-operative period during the shunt procedure, 3 weeks after the kaolin injection. The initial allocation of the remaining 34 animals was 10 controls, 11 low dose AXOKINE ® and 13 high dose AXOKINE ®. Eleven more rats died 33 to 41 days after kaolin injection (12 to 20 days after shunting). These were unevenly distributed amongst the experimental arms. Five surviving rats were subsequently excluded based on histological evidence of ventriculitis. The rats subjected to the AXOKINE ® treatment experienced a significant higher mortality rate than controls ($p < 0.02$) (See Table 4).

Table 4: Mortality and morbidity of shunted hydrocephalic rats treated with AXOKINE ®.

	Starting sample	Deaths	Exclusions	Final Sample size
Control	10	2	1	7
Low dose AXOKINE ®	11	5	2	4
High dose AXOKINE ®	13	4	2	7
Total	34	11	5	18

The ventricle size index determined prior to shunting of the rats that died in the low dose AXOKINE ® group was higher at 0.71 +/- 0.04 compared to 0.68 +/- 0.01 for the controls and 0.38 +/- 0.06 for the high dose AXOKINE ® group. All behavioral assessments will be reported based on animals that survived the entire experiment and were not excluded due to ventriculitis.

The ventricle size ratio (Figure 15) was not significantly different between the three groups at either time point. The change in ventricular size however was significant for the high dose AXOKINE ®-treated rats.

The weight gains presented in Figure 16 show a significant difference after the introduction of AXOKINE ® treatment.

Spontaneous activity data is presented in Table 5. Only the vertical count at pre-kaolin and in the third week of treatment (treatment-3) demonstrated any significant differences. This could be a random variation. The counts for the fourth week of the experiment show decrease for all groups. This period follows the surgical intervention and the change may be related to animal discomfort.

The roller assessments performed at a constant speed are shown in Figure 17. The times recorded represent the rats ability to stay on the roller; no significant differences were observed between groups.

The swimming results are represented in Figures 18, 19 and 20. The motor performance is assessed by timing the rats as they swim 1.5 m (Figure 18).

The cognitive functions of memory and learning can be inferred by looking at time needed to find the submerged platform. Figure 19 represents all times for both groups for the three trials in a single day. The rats are expected to improve their performance as they get older. This improvement can be observed for the first two weeks following kaolin injection for all three groups. However as the hydrocephalus progresses their performance should regress. After the third week the AXOKINE ®-treated rats (low and high dose) have longer times compared to controls. Upon initiation of treatment in week 4, the control rats show little or no improvement in an already superior performance. The low dose AXOKINE ® -treated rats show mild improvement with treatment but not the high dose AXOKINE ® -treated rats. This superior performance by the control rats is illustrated more clearly in Figures 20 and 21, which correspond to the first trial time of the day and the mean time of the day respectively.

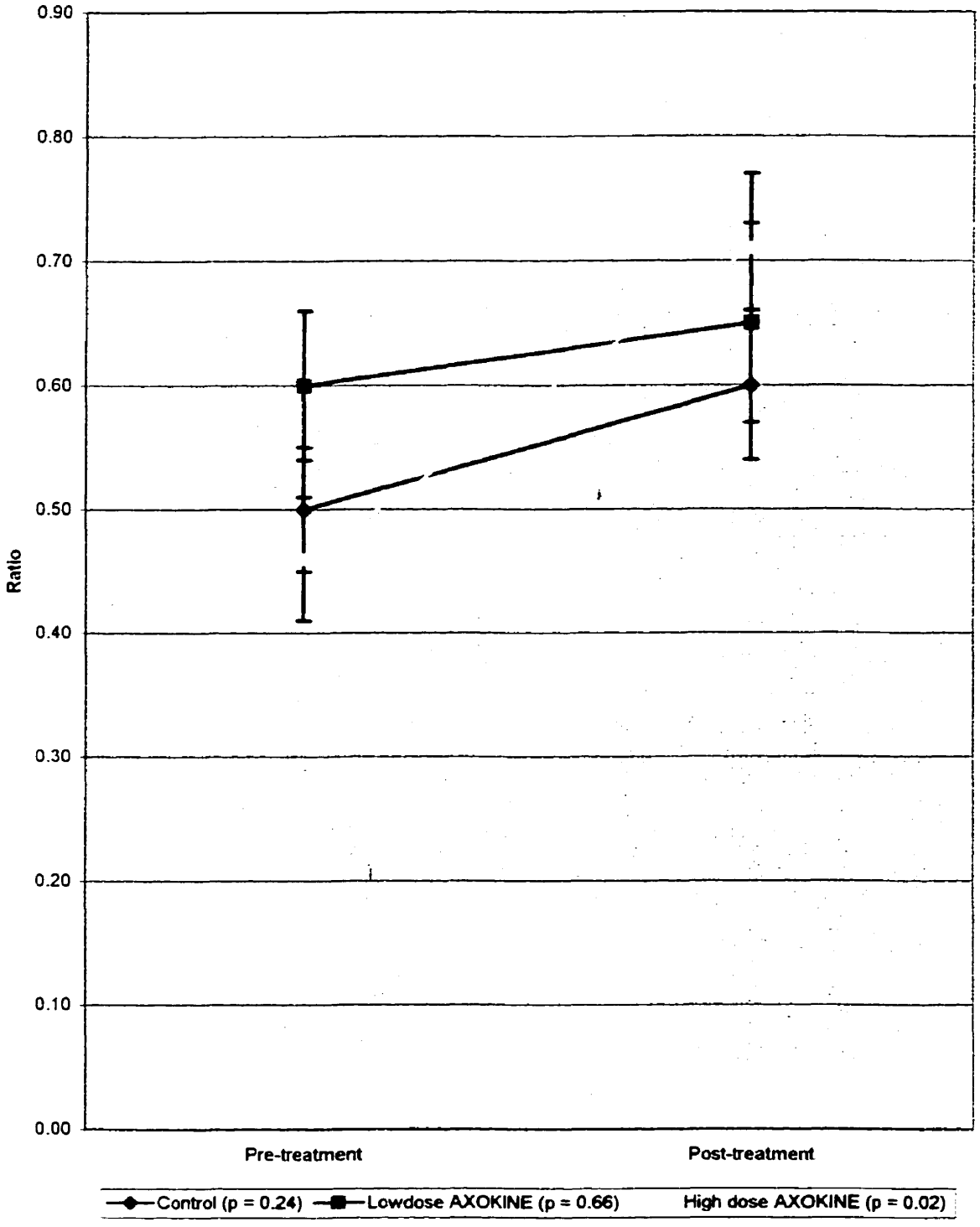

Figure 15: Ventricular size index changes before and after treatment. Significant change for high dose AXOKINE group, p = 0.02

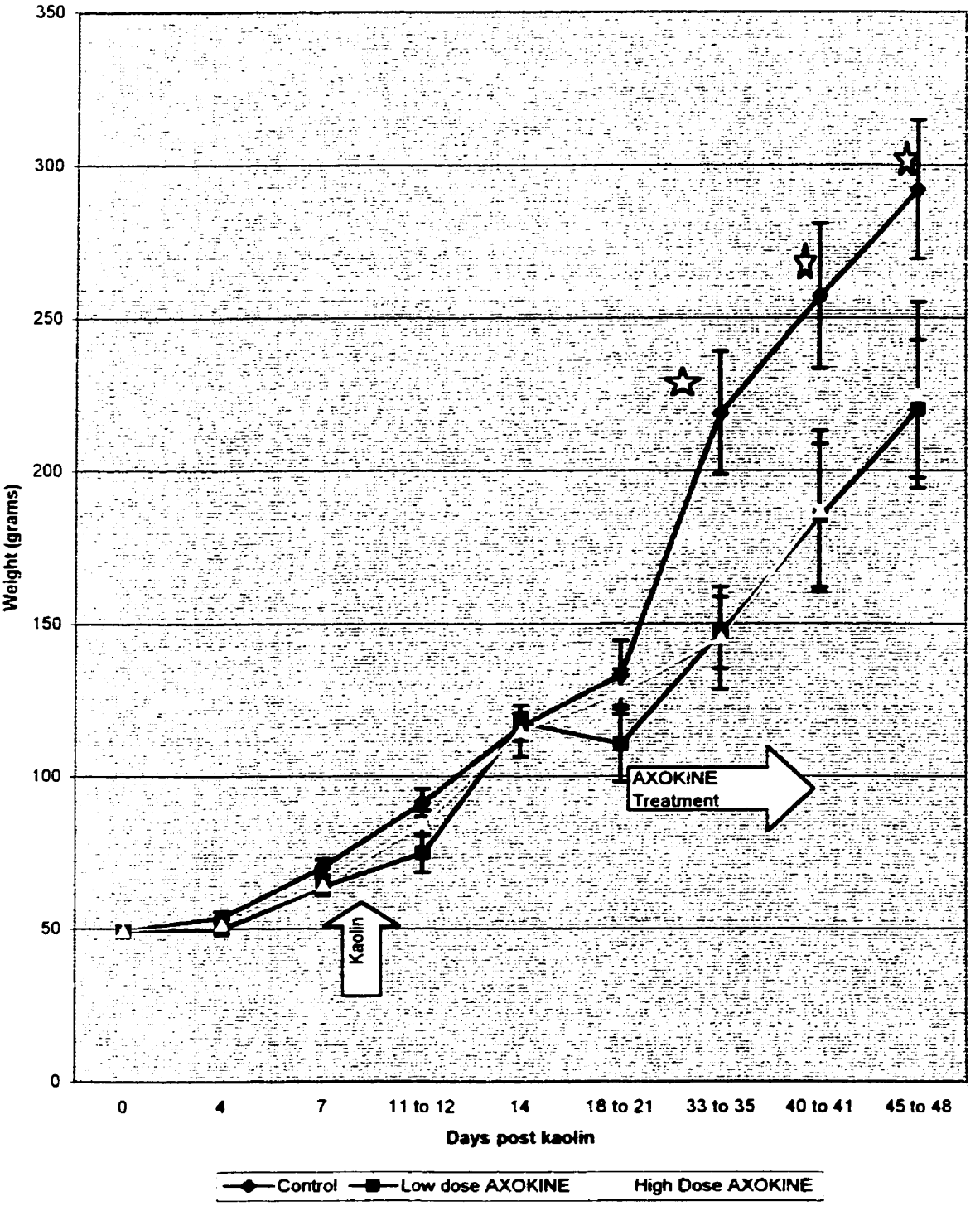

Figure 16: Weight gain for the AXOKINE experiment in relation to kaolin injection and treatment. stars = $p < 0.02$

Table 5a: Spontaneous activity total count

Time points	Control	Low dose AXOKINE *	High dose AXOKINE *
Week 0/Pre-kaolin	1626.9 +/- 164.0	1179.2 +/- 184.0	1522.29+/- 247.4
Week 1/ no treatment	1071.3 +/- 166.6	1161.2 +/- 161.2	1603.3 +/- 1191.8
Week 2/ no treatment	1704.3 +/- 174.8	2596.2 +/- 504.1	2162.4 +/- 410.0
Week 3/ no treatment	1957.1 +/- 432.5	1793.5 +/- 168.1	2592.3 +/- 517.3
Week 4/ with Treatment	1399.0 +/- 271.6	805.2 +/- 436.2	1684.3 +/- 664.1
Week 5/ with Treatment	1932.3 +/- 357.6	1870.8 +/- 607.3	1678.1 +/- 583.0
Week 6/ with Treatment	1980.9 +/- 203.6	1055.8 +/- 509.8	2153.3 +/- 708.1

no significant differences

Table 5b: Spontaneous activity ambulation count

Time points	Control	Low dose AXOKINE *	High dose AXOKINE *
Week 0/Pre-kaolin	918.3 +/- 107.0	608.0 +/- 103.7	808.0 +/- 168.8
Week 1/ no treatment	486.3 +/- 113.5	549.2 +/- 82.2	825.3 +/- 128.0
Week 2/ no treatment	920.6 +/- 131.9	1555.0 +/- 378.5	1380.7 +/- 295.2
Week 3/ no treatment	1177.6 +/- 281.4	1076.2 +/- 123.9	1828.8 +/- 408.5
Week 4/ with Treatment	917.9 +/- 202.9	538.8 +/- 316.3	1130.0 +/- 539.1
Week 5/ with Treatment	1335.0 +/- 321.4	1294.5 +/- 487.2	1116.3 +/- 480.5
Week 6/ with Treatment	1365.6 +/- 175.9	708.8 +/- 473.0*	1621.6 +/- 587.1

* significant difference between low dose AXOKINE * and both high dose AXOKINE * and control, p = 0.02 in week 6 only.

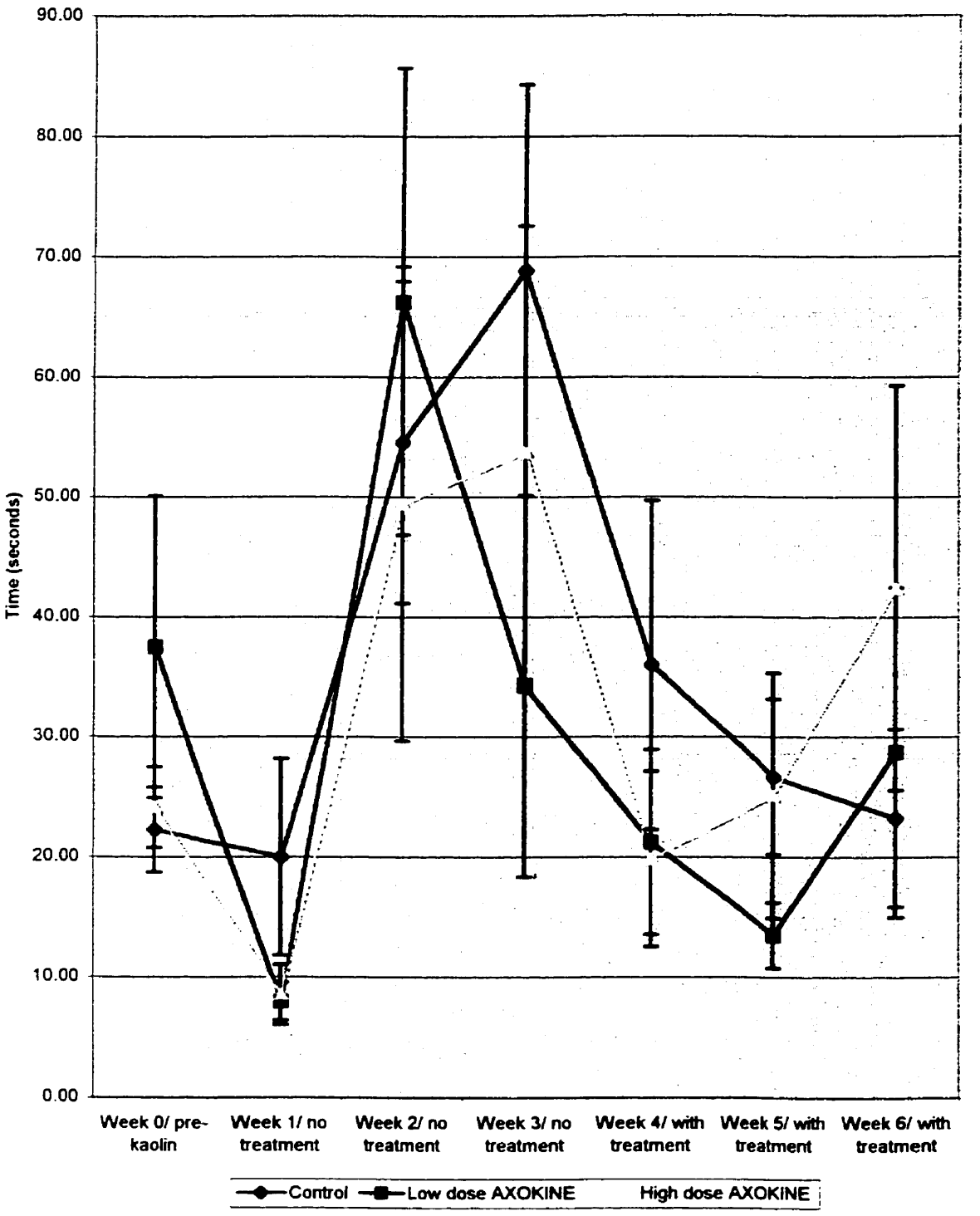

Figure 17: Endurance (time) of rat walking on roller at constant speed (5 rpm), no significant differences.

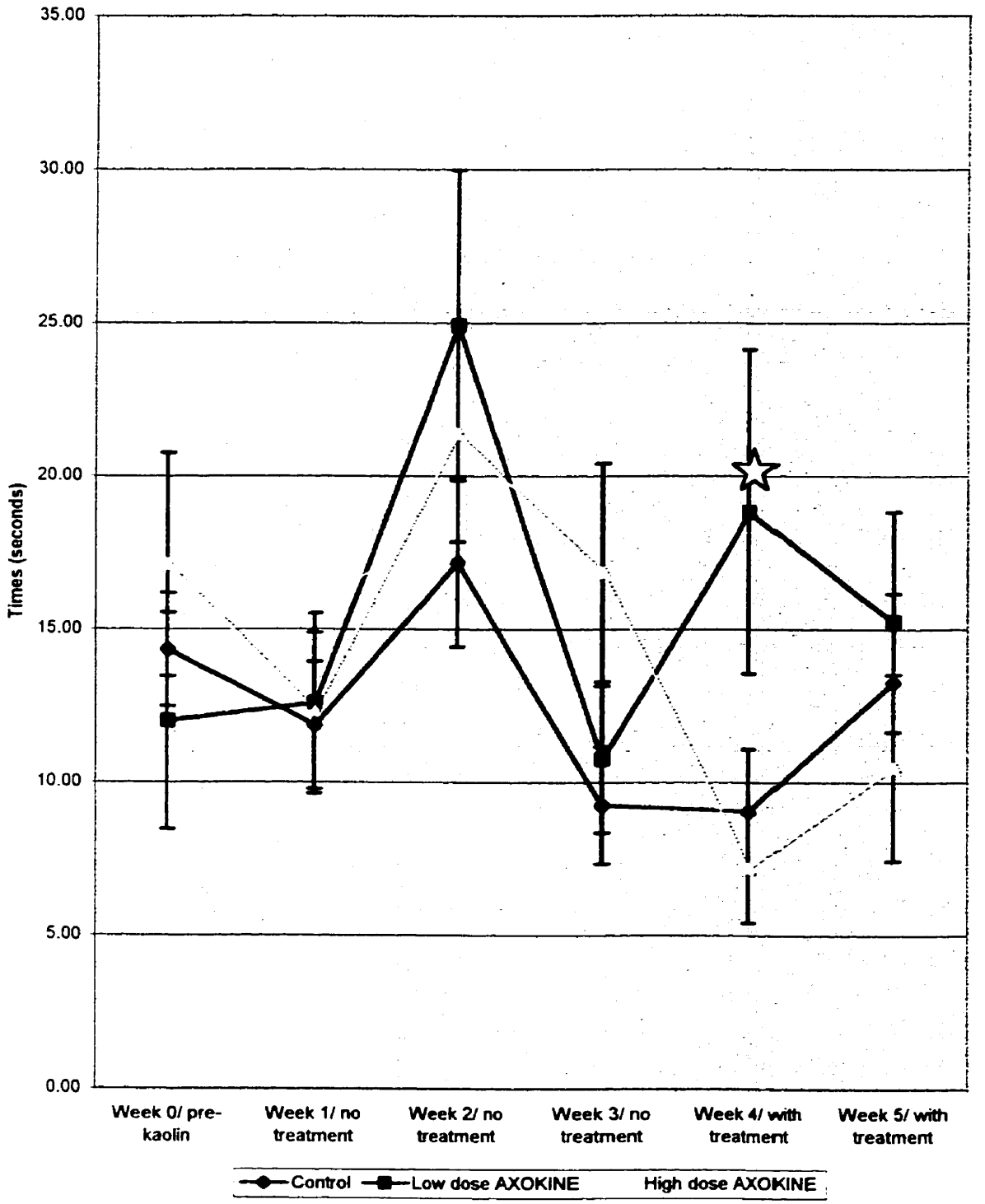

Figure 18: Time required to swim 1.5 m in the AXOKINE experiment, star = $p < 0.02$ Low dose AXOKINE versus others

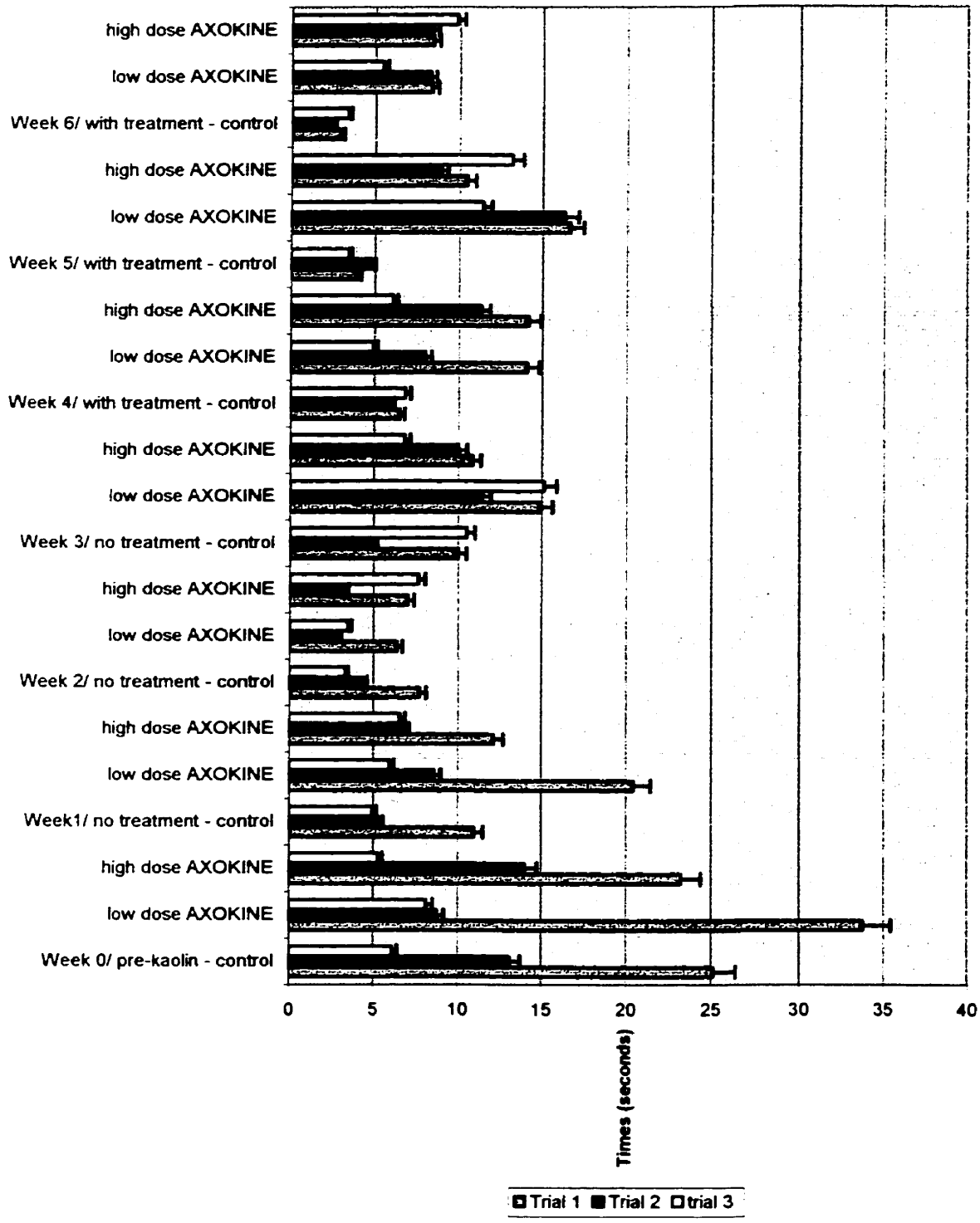

Figure 19: Time required to find submerged platform, each experimental arm is depicted weekly with all three trials of the day.

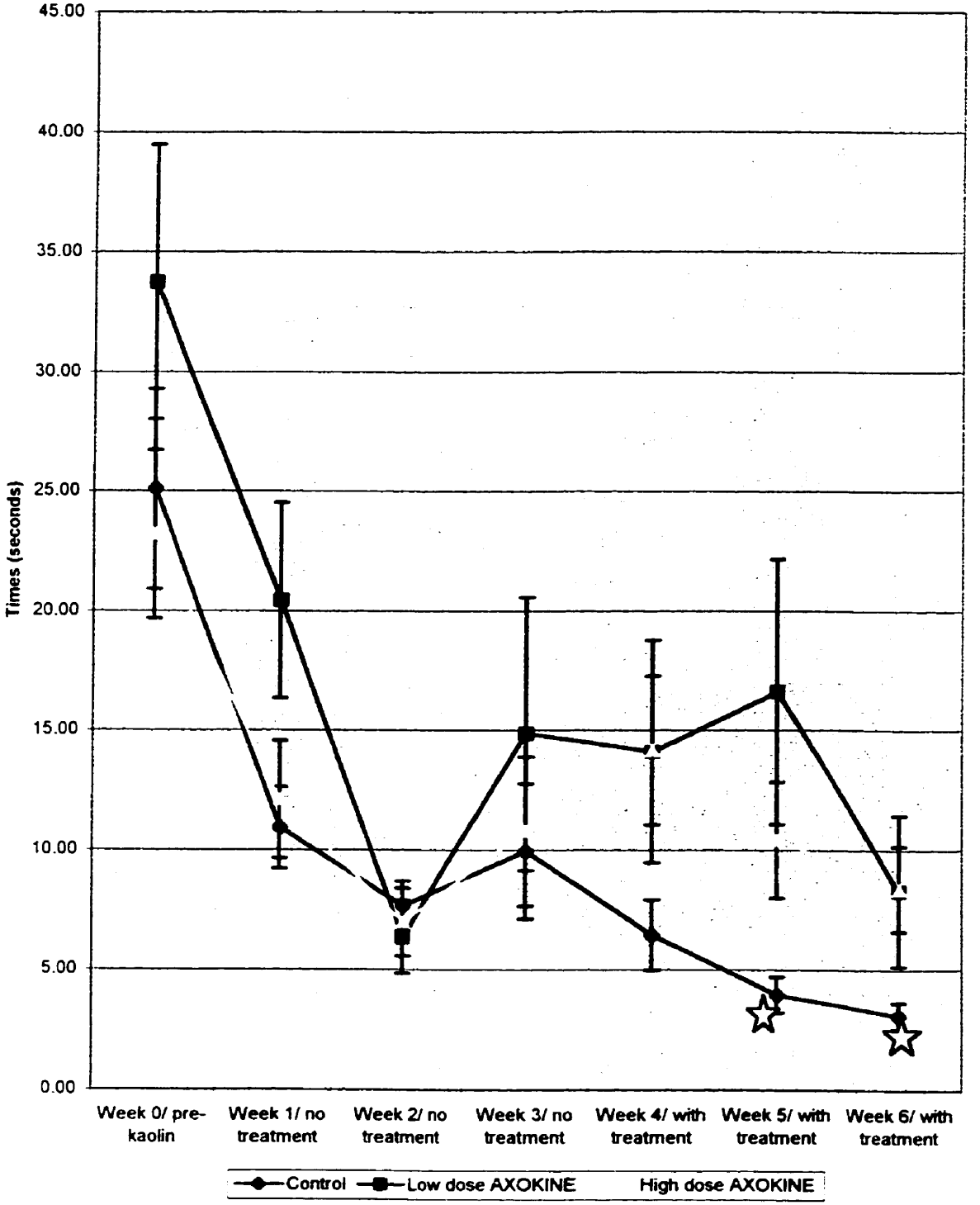

Figure 20: Swimming times for the first trial of the day, star = $p < 0.04$, control versus both treatment groups.

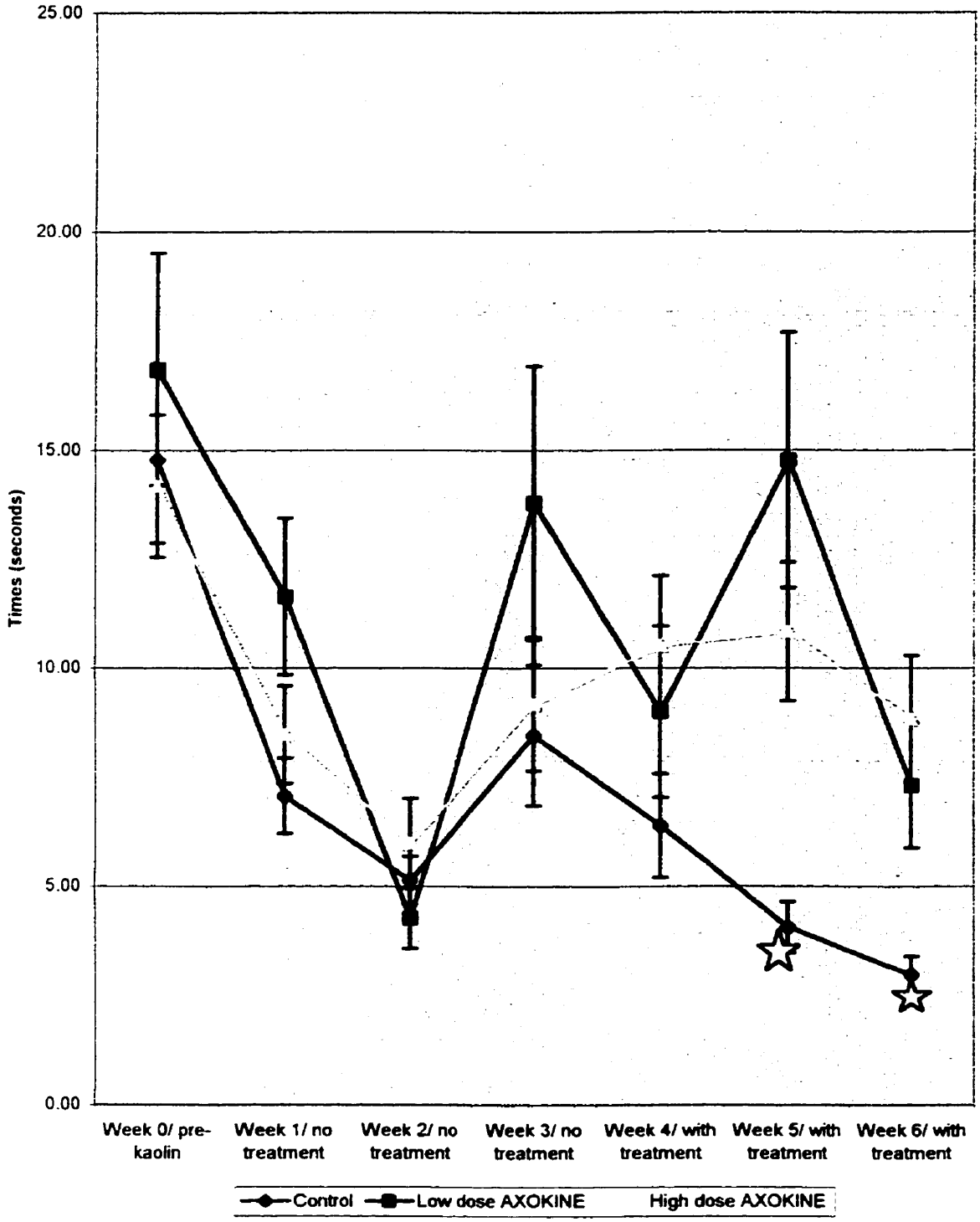

Figure 21: Swimming times for the mean of the three trials in the day, star = $p < 0.01$, control versus both treatment groups.

Microscopic observations of coronal brain slices at the level of the optic chiasm were performed on 23 rats. H & E slides were used to identify rats with evidence of ventriculitis. Ventriculitis was deemed significant if neutrophils and lymphocytes were observed in the ventricular system. The location and amount of kaolin was also noted. Eight rats demonstrated small lesions related to the tubing insertion. Two rats had striatal hematoma and six had either epidural or subdural hematoma measuring one to two mm in thickness. MBP immunolabeled slides were used to measure the thickness of the corpus callosum (CC). Table 6 summarizes the results of the corpus callosum thickness for all three groups. The only significant difference noted was a thinner corpus callosum for the low dose AXOKINE ® -treated rats. This result was unexpected. The GFAP slides were used to generate optical densitometric data (Table 7). The significant differences were found with the AXOKINE ® -treated animals showing a higher astrocyte reactivity. The neurofilament immunohistochemical staining did not show any qualitative differences between controls and AXOKINE ® -treated group.

Table 6: Corpus callosum thickness measurements from MBP labeled slides

Corpus callosum thickness (μm)	Midline measurement	Lateral measurement
Control	350.0 +/- 92.2	362.5 +/- 79.0
Low dose AXOKINE ®	255.0 +/- 88.2	216.7 +/- 16.7 *
High dose AXOKINE ®	256.2 +/- 35.6	378.1 +/- 66.4

* = significant differences between low dose and both high dose and control, $p < 0.04$.

Table 7: GFAP optical densitometry readings (arbitrary units)

Anatomical location	Corpus callosum	Fimbria	Cortex
Control	0.377 +/- 0.008	0.477 +/- 0.008	0.451 +/- 0.015
Low dose AXOKINE ®	0.371 +/- 0.004	0.519 +/- 0.022	0.541 +/- 0.028 *
High dose AXOKINE ®	0.379 +/- 0.004	0.491 +/- 0.014	0.516 +/- 0.020 *

* = difference between control and AXOKINE ® -treated cortex, $p < 0.01$

4.0 Discussion

The two methods of experimental pharmacologic treatment of rats with kaolin-induced hydrocephalus, nimodipine and AXOKINE ®, will be discussed separately.

4.1 Nimodipine experiment

Was nimodipine successful in minimizing brain damage and improving functional outcome associated with experimental hydrocephalus in young rats?

Weight gain, increase in ventricle size ratio, and mortality of the control and nimodipine groups were similar prior to and following treatment. Therefore differences in behavioral outcome are likely related to a treatment effect on brain and not a difference between the initial severity of hydrocephalus between groups.

The roller test demonstrated a benefit of treatment with nimodipine. The rats had more endurance at a constant speed after two weeks of treatment with nimodipine and were more adept at dealing with the constant acceleration test. The time required for the rats to swim a specific distance (1.5 m) also suggested superior motor abilities of the nimodipine-treated rats. Together these indicate a certain degree of protection in the motor function of the nimodipine-treated rats.

The absence of differences in spontaneous activity could be an artifact. Although the rats were tested in the same manner for technical reasons, they were not necessarily assessed at the same time of day. Therefore diurnal variability might mask a difference. Other tests were performed around the same period. It is conceivable that some rats received more stimulation prior to being placed into the chamber for spontaneous activity monitoring. The level of stimulation was controlled by having the same technician perform the assessment and only during the daytime. The other possibility may be related to the lack of sensitivity of this assessment method for the particular symptoms exhibited by hydrocephalic rats.

Swimming maze assessment focused on learning and memory but was also affected by the motor ability of the rats. We showed the nimodipine-treated group to have a better motor performance in swimming. The first trial of the day calls upon the ability to remember the performance from the previous week and the cognitive ability to form a search strategy. The mean time and particularly the improvement during the day reflect the cognitive ability of the rat. The ability to learn a new task can be quantified by comparing the improvement during a given day. The nimodipine-treated rats exhibited the ability to continue learning and formulate search strategies despite their ventriculomegaly. This was illustrated in Figure 9 by improvement of the times recorded from the first to the third trial in a single day.

The corpus callosum of nimodipine-treated animals was thicker when compared to the control animals. A thicker corpus callosum is interpreted as preservation of axons and myelin [39]. The neurofilament labeling provided identification of axons. Beaded or varicose nerve fibers can be the result of pathological change elicited with mild stretching [111]. Stretching will cause local constriction of the axon and subsequently affect axoplasmic fluid and soluble components. This constriction will therefore cause neighboring expansion of the axon, which can be seen microscopically. Immunohistochemical staining with neurofilament revealed no significant differences between control and nimodipine groups. This lack of significant differences may be attributed to numerous factors. The subjective nature of the observations and the tissue sampling may have played a role. The degree of edema was not measured directly, but could influence the proximity of axons being observed. The state of the tissue prior to fixation would impact on our quantitative measurements. The quality of the tissue was controlled by having the animals euthanized on the same day and using standard tissue manipulations.

The optical densitometric readings from tissues labeled with anti-GFAP to demonstrate reactive gliosis [41] revealed mild differences in the corpus callosum suggesting greater level of reaction in the nimodipine-treated rats. This reactive gliosis seems to be associated with preservation of axons and myelin resulting in a

thicker corpus callosum. Whether this is of importance to the apparent protective effect of nimodipine or simply coincidence is not known.

The protective role of nimodipine in hydrocephalic rats seems to be significant. Nimodipine-treated rats had less motor dysfunction and their ability to learn new tasks was preserved. This functional outcome was correlated with histological preservation of corpus callosum thickness. Speculation on the mechanism of neuroprotection is based on the Ca^{2+} channel blocking property of nimodipine and the subsequent reduction of Ca^{2+} influx. The presence of L-type Ca^{2+} channels in white matter has been demonstrated to be up-regulated with ischemia[156]. The increased calpain activity observed in traumatic brain injuries [109] and in our data (Del Bigio et al, 2000 pending publication) may also play a role in axonal and myelin damage. The specific order of events in this cascade are unclear but involvement of Ca^{2+} influx and μ–calpain activation seem associated with damage to myelin and axons.

The potential for increased cerebral blood flow with nimodipine treatment must also be factored into the cytological environment. The neuroprotection may be a reflection of ischemia prevention. The clinical value of nimodipine for ischemic brain has been demonstrated [3;101;108;119;120]. Pathophysiological mechanisms in hydrocephalus certainly include ischemia which, in our experiment, seems to be minimized by nimodipine.

The ability of nimodipine to penetrate rat brain combined with specific effect on neurons by single cell recording of depolarization [133] support the hypothesis of neuronal protection based on inhibition of calcium influx. Prevention of ischemia by increasing cerebral flow would also render cerebral protection. The lack of control in our experiment for cerebral blood flow prohibits any specific conclusion regarding mechanism of protection. Treatment with various doses could provide some information regarding the effect of vascular smooth muscle versus neuronal or glial on neuronal protection. This would assume different dose-response curves for vascular smooth muscle, neurons and glial cells.

4.2 AXOKINE ® experiment

Was AXOKINE ® successful in promoting brain recovery and improving functional outcome following shunting of young hydrocephalic rat brain?

Failure to demonstrate any improvement in functional outcome with AXOKINE ® treatment can be attributed to several factors. The AXOKINE ® treated rats had a high attrition rate, which favored the control group. Both low dose and high dose AXOKINE ®-treated groups had 46 and 63% drop-out rates respectively compared to 30% for the controls. A higher overall mortality compared to the nimodipine experiment was expected since the duration of the experiment was seven weeks. The animals were allowed to develop hydrocephalus for three weeks prior to treatment. The surgical procedure contributed to more cerebral trauma compared to the nimodipine experiment, two striatal injuries proven by histological examination and six hematoma were found at the time of dissection. Brains of rats dying unexpectedly were generally autolyzed and therefore not analyzed microscopically. The surgery may have caused more infection despite the administration of prophylactic antibiotics. Five rats had evidence of ventriculitis based on histological examination. Perhaps this could have been reduced by a longer period of antibiotic administration.

A second reason for failure may be related to the different degree of ventriculomegaly for each group. The unsuccessful shunting of the high dose AXOKINE ® group resulted in a significant increase in their ventricular size ratio. The expectation with shunting is to decrease or stabilize the ventricle size [37]. The shunt failure may be attributable to infection and blockage. Shunting and inserting a pump to deliver AXOKINE ® into the ventricle of young rats may be beyond technical feasibility.

Weight gain was also different when comparing the controls with either the low or high dose AXOKINE ®. The cytokine-like effects of CNTF were expected to be less with AXOKINE ® [68]. Nevertheless both AXOKINE ®-treated groups gained less weight during treatment compared to controls. This could be related to the ventriculomegaly which was more pronounced in the high dose AXOKINE ® group only. The other harmful side effect related to cytokine-like side effect may have been suppression of the rat's ability to fight infection. The increased infection may have led to the higher mortality. The selection of a different neurotrophic factor could have minimized this side effect.

Based on the evidence reported, we are unable to conclude that there is any beneficial effect of AXOKINE ®. The confounding factors previously identified (poor weight gain, high mortality and failure of shunting) might have obscured any functional differences that could be attributed to this treatment. Other causes

for our failure to demonstrate improvement may be related to factors such as ineffective drug. This could in part explain why the cytokine-like side effects were still manifested. Repeating the experiment with larger sample size and greater attention to surgical details and hand feeding may show some advantage. However, no aspect of the data indicates a potential for treatment benefit.

A second reason for failure may be related to the different degree of ventriculomegaly for each group. The unsuccessful shunting of the high dose AXOKINE ® group resulted in a significant increase in their ventricular size ratio. The expectation with shunting is to decrease or stabilize the ventricle size [37]. The shunt failure may be attributable to infection and blockage. Shunting and inserting a pump to deliver AXOKINE ® into the ventricle of young rats may be beyond technical feasibility.

Weight gain was also different when comparing the controls with either the low or high dose AXOKINE ®. The poor weight gain can be related to the appetite suppression secondary to high dose CNTF direct effect on the hypothalamic feeding centers [161]. Nevertheless both AXOKINE ®-treated groups gained less weight during treatment compared to controls. This could be related to the ventriculomegaly which was more pronounced in the high dose AXOKINE ® group only. The other harmful side effect related to CNTF administration may have been suppression of the rat's ability to fight infection. The increased infection may have led to the higher mortality. The selection of a different neurotrophic factor could have minimized this side effect.

Based on the evidence reported, we are unable to conclude that there is any beneficial effect of AXOKINE ®. The confounding factors previously identified (poor weight gain, high mortality and failure of shunting) might have obscured

any functional differences that could be attributed to this treatment. Other causes for our failure to demonstrate improvement may be related to factors such as ineffective drug. This could in part explain why the cytokine-like side effects were still manifested. Repeating the experiment with larger sample size and greater attention to surgical details and hand feeding may show some advantage. However, no aspect of the data indicates a potential for treatment benefit.

4.3 Conclusion and Future Directions

In conclusion, the results of the nimodipine experiment are sufficiently robust to warrant additional experiments which would determine whether it has potential as an adjunctive therapy for hydrocephalus. These would include replication of the data in another animal model and delineation of the mechanism of protection prior to any clinical trials. Nimodipine is already in clinical use for prevention of cerebral ischemia in patients with aneurysmal subarachnoid hemorrhage, and therefore has demonstrated its safety in human subjects. Nimodipine has been administered to a pediatric population as an adjunctive therapy for drug-resistant childhood epilepsy [91;117] and in the treatment of pediatric migraine [13]. Shahar et al, in a review of Ca^{2+} channel blocker used in pediatric emergency care, refer to nimodipine as potentially effective in reducing brain damage following trauma [135]. Clinical investigation on pediatric patients with hydrocephalus is the extension of this work, but the adult population could also benefit from nimodipine. Future studies would require a lengthy follow-up period in order to detect subtle improvements. The objective would be to supplement and not replace CSF diversion for some patients and minimize the neurological deficits resulting from hydrocephalus.

Post-shunt regeneration of axons and neurons and specifically the role of neurotrophins in the hydrocephalic brain needs further clarification. The concept seems sound but the application still requires choosing the right factor and an appropriate delivery method. Unfortunately neurotrophic agents in general do not cross the blood brain barrier [31;121]. With the development of new neurotrophins this avenue of research will need to be revisited.

The frustration encountered by hydrocephalic patients and their families needs to be alleviated. The limited armamentarium available to the neurosurgeon fuels this frustration. Drug therapy directed at protecting and regenerating damaged neurons in hydrocephalus constitutes a new avenue of investigation. This series of experiments, whose ultimate goal is to prevent or reverse all damage caused by hydrocephalus, is only the first step.

5.0 References

1. *International randomised controlled trial of acetazolamide and furosimide in posthaemorrhagic ventricular dilatation in infancy.* **Lancet 352:** 433-440, 1998

2. Ahlberg, J., Norlen, L., Blomstrand, C., and Wikkelso, C.: *Outcome of shunt operation on urinary incontinence in normal pressure hydrocephalus predicted by lumbar puncture.* **J.Neurol Neurosurg.Psychiatry 51:** 105-108, 1988

3. Allen, G. S., Ahn, H. S., Preziosi, T. J., Battye, R., Boone, S. C., Chou, S. N., Kelly, D. L., Weir, B. K., Crabbe, R. A., Lavik, P. J., Rosenbloom, S. B., Dorsey, F. C., Ingram, C. R., Mellits, D. E., Bertsch, L. A., Boisvert, D. P., Hundley, M. B., Johnson, R. K., Strom, J. A., and Transou, C. R.: *Cerebral arterial spasm--a controlled trial of nimodipine in patients with subarachnoid hemorrhage.* **N.Engl.J.Med. 308:** 619-624, 17-3-1983

4. Andersen, C.: *In vivo estimation of water content in cerebral white matter of brain tumour patients and normal individuals: towards a quantitative brain oedema definition.* **Acta Neurochir.Wien. 139:** 249-255, 1997

5. Anderson, B.: *Relief of akinetic mutism from obstructive hydrocephalus using bromocriptine and ephedrine. Case report.* **J.Neurosurg. 76:** 152-155, 1992

6. Anderson, K. D., Panayotatos, N., Corcoran, T. L., Lindsay, R. M., and Wiegand, S. J.: *Ciliary neurotrophic factor protects striatal output neurons in an animal model of Huntington disease.* **Proc.Natl.Acad.Sci.U.S.A. 93:** 7346-7351, 9-7-1996

7. Bahr, B. A., Abai, B., Gall, C. M., Vanderklish, P. W., Hoffman, K. B., and Lynch, G.: *Induction of beta-amyloid-containing polypeptides in hippocampus: evidence for a concomitant loss of synaptic proteins and interactions with an excitotoxin.* **Exp.Neurol 129:** 81-94, 1994

8. Balentine, J. D.: *Spinal cord trauma: in search of the meaning of granular axoplasm and vesicular myelin.* **J.Neuropathol.Exp.Neurol. 47:** 77-92, 1988

9. Ball, M. J.: *Neurofibrillary tangles in the dementia of "normal pressure" hydrocephalus.* **Can.J.Neurol.Sci. 3:** 227-235, 1976

10. Ball, M. J. and Vis, C. L.: *Relationship of gramulovacuolar degeneration in hippocampal neurones to aging and to dementia in normal-pressure hydrocephalics.* **J.Gerontol. 33:** 815-824, 1978

11. Bannister, C. M. and Mundy, J. E.: *Some scanning electron microscopic observations of the ependymal surface of the ventricles of hydrocephalic Hy3 mice and a human infant.* **Acta Neurochir.Wien. 46:** 159-168, 1979

12. Baratti, C., Barnett, A. S., and Pierpaoli, C.: *Comparative MR imaging study of brain maturation in kittens with T1, T2, and the trace of the diffusion tensor.* **Radiology 210:** 133-142, 1999

13. Battistella, P. A., Ruffilli, R., Moro, R., Fabiani, M., Bertoli, S., Antolini, A., and Zacchello, F.: *A placebo-controlled crossover trial of nimodipine in pediatric migraine.* **Headache. 30:** 264-268, 1990

14. Beilke, M. A., Collins, Lech C., and Sohnle, P. G.: *Effects of dimethyl sulfoxide on the oxidative function of human neutrophils.* **J.Lab.Clin.Med 110:** 91-96, 1987

15. Bellemann, P., Schade, A., and Towart, R.: *Dihydropyridine receptor in rat brain labeled with [3H]nimodipine.* **Proc.Natl.Acad.Sci.U.S.A. 80:** 2356-2360, 1983

16. Benowitz, L. I. and Perrone, Bizzozero NI: *The expression of GAP-43 in relation to neuronal growth and plasticity: when, where, how, and why?* **Prog.Brain Res 89:** 69-87, 1991

17. Benowitz, L. I. and Routtenberg, A.: *GAP-43: an intrinsic determinant of neuronal development and plasticity.* **Trends.Neurosci. 20:** 84-91, 1997

18. Berker, E., Goldstein, G., Lorber, J., Priestley, B., and Smith, A.: *Reciprocal neurological developments of twins discordant for hydrocephalus.* **Dev.Med Child Neurol 34:** 623-632, 1992

19. Braun, K. P., de Graaf, R A, Vandertop, W. P., Gooskens, R. H., Tulleken, C. A., and Nicolay, K.: *In Vivo ^{1}H MR Spectroscopic Imaging and Diffusion Weighted MRI in Experimental Hydrocephalus.* **Magnetic Resonance in Medicine 40:** 832-839, 1998

20. Bret, P., Chazal, J., Janny, P., Renaud, B., Tommasi, M., Lemaire, J. J., Bret, M., and Jouvet, A.: *[Chronic hydrocephalus in adults].* **Neurochirurgie. 36 Suppl 1:** 1-159, 1990

21. Bruni, J. E., Del Bigio, M. R., and Clattenburg, R. E.: *Ependyma: normal and pathological. A review of the literature.* **Brain Res. 356:** 1-19, 1985

22. Choi, D. W.: *Glutamate neurotoxicity and diseases of the nervous system.* **Neuron 1:** 623-634, 1988

23. Clatterbuck, R. E., Price, D. L., and Koliatsos, V. E.: *Further characterization of the effects of brain-derived neurotrophic factor and ciliary neurotrophic factor on axotomized neonatal and adult mammalian motor neurons.* **J.Comp.Neurol. 342:** 45-56, 1-4-1994

24. Collins, P.: *Experimental obstructive hydrocephalus in the rat: a scanning electron microscopic study.* **Neuropathol.Appl.Neurobiol. 5:** 457-468, 1979

25. Cook, J. L., Irias, Donaghey S., and Deininger, P. L.: *Regulation of rodent myelin proteolipid protein gene expression.* **Neurosci.Lett. 137:** 56-60, 16-3-1992

26. Croall, D. E. and DeMartino, G. N.: *Calcium-activated neutral protease (calpain) system: structure, function, and regulation.* **Physiol.Rev. 71:** 813-847, 1991

27. Cuello, A. C.: *Experimental neurotrophic factor therapy leads to cortical synaptic remodeling and compensates for behavioral deficits.* **J.Psychiatry Neurosci. 22:** 46-55, 1997

28. Curran, T. and Lang, A. E.: *Parkinsonian syndromes associated with hydrocephalus: case reports, a review of the literature, and pathophysiological hypotheses.* **Mov.Disord. 9:** 508-520, 1994

29. da Silva, M. C., Michowicz, S., Drake, J. M., Chumas, P. D., and Tuor, U. I.: *Reduced local cerebral blood flow in periventricular white matter in*

experimental neonatal hydrocephalus-restoration with CSF shunting. **J.Cereb.Blood Flow Metab. 15:** 1057-1065, 1995

30. Davidoff, L. M.: *Treatment of hydrocephalus. Historical review and description of a new method.* **Arch.Surg. 18:** 1737-1762, 1929

31. Deglon, N., Heyd, B., Tan, S. A., Joseph, J. M., Zurn, A. D., and Aebischer, P.: *Central nervous system delivery of recombinant ciliary neurotrophic factor by polymer encapsulated differentiated C2C12 myoblasts.* **Hum.Gene Ther. 7:** 2135-2146, 10-11-1996

32. Del Bigio, M. R.: *Hydrocephalus and periventricular white matter water content [letter].* **J.Neurosurg. 67:** 955-956, 1987

33. Del Bigio, M. R.: *Neuropathological changes caused by hydrocephalus.* **Acta Neuropathol.Berl. 85:** 573-585, 1993

34. Del Bigio, M. R. and Bruni, J. E.: *Periventricular pathology in hydrocephalic rabbits before and after shunting.* **Acta Neuropathol.Berl. 77:** 186-195, 1988

35. Del Bigio, M. R., Bruni, J. E., and Vriend, J. P.: *Monoamine neurotransmitters and their metabolites in the mature rabbit brain following induction of hydrocephalus.* **Neurochem.Res. 23:** 1379-1386, 1998

36. Del Bigio, M. R., Cardoso, E. R., and Halliday, W. C.: *Neuropathological changes in chronic adult hydrocephalus: cortical biopsies and autopsy findings.* **Can.J.Neurol.Sci. 24:** 121-126, 1997

37. Del Bigio, M. R., Crook, C. R., and Buist, R.: *Magnetic resonance imaging and behavioral analysis of immature rats with kaolin-induced hydrocephalus: pre- and postshunting observations.* **Exp.Neurol. 148:** 256-264, 1997

38. Del Bigio, M. R., da Silva, M. C., Drake, J. M., and Tuor, U. I.: *Acute and chronic cerebral white matter damage in neonatal hydrocephalus.* **Can.J.Neurol.Sci. 21:** 299-305, 1994

39. Del Bigio, M. R., Kanfer, J. N., and Zhang, Y. W.: *Myelination delay in the cerebral white matter of immature rats with kaolin-induced hydrocephalus is reversible.* **J.Neuropathol.Exp.Neurol. 56:** 1053-1066, 1997

40. Del Bigio, M. R. and Vriend, J. P.: *Monoamine neurotransmitters and amino acids in the cerebrum and striatum of immature rats with kaolin-induced hydrocephalus.* **Brain Res. 798:** 119-126, 6-7-1998

41. Del Bigio, M. R. and Zhang, Y. W.: *Cell death, axonal damage, and cell birth in the immature rat brain following induction of hydrocephalus.* **Exp.Neurol. 154:** 157-169, 1998

42. Dennis, M., Jacennik, B., and Barnes, M. A.: *The content of narrative discourse in children and adolescents after early-onset hydrocephalus and in normally developing age peers.* **Brain Lang. 46:** 129-165, 1994

43. Deshpande, J. K., Siesjo, B. K., and Wieloch, T.: *Calcium accumulation and neuronal damage in the rat hippocampus following cerebral ischemia.* **J.Cereb.Blood Flow Metab. 7:** 89-95, 1987

44. Drake, J. M., da Silva, M. C., and Rutka, J. T.: *Functional obstruction of an antisiphon device by raised tissue capsule pressure.* **Neurosurgery 32:** 137-139, 1993

45. Drake, J. M., Kestle, J. R., Milner, R., Cinalli, G., Boop, F., Piatt, J., Haines, S., Schiff, S. J., Cochrane, D. D., Steinbok, P., and MacNeil, N.: *Randomized trial of cerebrospinal fluid shunt valve design in pediatric hydrocephalus.* **Neurosurgery 43:** 294-303, 1998

46. Dubois, Dalcq M. and Armstrong, R.: *The cellular and molecular events of central nervous system remyelination.* **Bioessays 12:** 569-576, 1990

47. Edwards, M. S., Harrison, M. R., Halks, Miller M., Nakayama, D. K., Berger, M. S., Glick, P. L., and Chinn, D. H.: *Kaolin-induced congenital hydrocephalus in utero in fetal lambs and rhesus monkeys.* **J.Neurosurg. 60:** 115-122, 1984

48. Ellison, J. A., Scully, S. A., and de Vellis, J.: *Evidence for neuronal regulation of oligodendrocyte development: cellular localization of platelet-derived growth factor alpha receptor and A-chain mRNA during cerebral cortex development in the rat.* **J.Neurosci.Res. 45:** 28-39, 1-7-1996

49. Emerich, D. F., Bruhn, S., Chu, Y., and Kordower, J. H.: *Cellular delivery of CNTF but not NT-4/5 prevents degeneration of striatal neurons in a rodent model of Huntington's disease.* **Cell Transplant. 7:** 213-225, 1998

50. Eriksdotter, Jonhagen M., Nordberg, A., Amberla, K., Backman, L., Ebendal, T., Meyerson, B., Olson, L., Seiger, Shigeta, M., Theodorsson, E., Viitanen, M., Winblad, B., and Wahlund, L. O.: *Intracerebroventricular infusion of nerve growth factor in three patients with Alzheimer's disease.* **Dement.Geriatr.Cogn.Disord. 9:** 246-257, 1998

51. Fan, K. J. and Pezeshkpour, G.: *Neurofibrillary tangles in association with congenital hydrocephalus.* **J.Natl.Med.Assoc. 79:** 1001-3,1006, 1987

52. Fisher, C. M.: *The clinical picture in occult hydrocephalus.* **Clin.Neurosurg. 24:** 270-284, 1977

53. Fishman, R. A and Greer, M.: *Experimental obstructive hydrocephalus. Changes in the cerebrum.* **Arch Neurol 8:** 156-161, 1963

54. Gadsdon, D. R., Variend, S., and Emery, J. L.: *Myelination of the corpus callosum. II. The effect of relief of hydrocephalus upon the processes of myelination.* **Z.Kinderchir.Grenzgeb. 28:** 314-321, 1979

55. Glees, P. and Voth, D.: *Clinical and ultrastructural observations of maturing human frontal cortex. Part I (Biopsy material of hydrocephalic infants).* **Neurosurg.Rev. 11:** 273-278, 1988

56. Goh, D., Minns, R. A., Hendry, G. M., Thambyayah, M., and Steers, A. J.: *Cerebrovascular resistive index assessed by duplex Doppler sonography and its relationship to intracranial pressure in infantile hydrocephalus.* **Pediatr.Radiol. 22:** 246-250, 1992

57. Goh, D., Minns, R. A., Pye, S. D., and Steers, A. J.: *Cerebral blood flow velocity changes after ventricular taps and ventriculoperitoneal shunting.* **Childs.Nerv.Syst. 7:** 452-457, 1991

58. Gopinath, G., Bhatia, R., and Gopinath, P. G.: *Ultrastructural observations in experimental hydrocephalus in the rabbit.* **J.Neurol.Sci. 43:** 333-334, 1979

59. Granholm, A. C., Albeck, D., Backman, C., Curtis, M., Ebendal, T., Friden, P., Henry, M., Hoffer, B., Kordower, J., Rose, G. M., Soderstrom, S., and Bartus, R. T.: *A non-invasive system for delivering neural growth factors across the blood-brain barrier: a review.* **Rev.Neurosci. 9:** 31-55, 1998

60. Grinspan, J. B., Stern, J. L., Franceschini, B., and Pleasure, D.: *Trophic effects of basic fibroblast growth factor (bFGF) on differentiated oligodendroglia: a mechanism for regeneration of the oligodendroglial lineage.* **J.Neurosci.Res. 36:** 672-680, 15-12-1993

61. Hakim, S., Venegas, J. G., and Burton, J. D.: *The physics of the cranial cavity, hydrocephalus and normal pressure hydrocephalus: mechanical interpretation and mathematical model.* **Surg.Neurol. 5:** 187-210, 1976

62. Hanigan, W. C., Morgan, A., Shaaban, A., and Bradle, P.: *Surgical treatment and long-term neurodevelopmental outcome for infants with idiopathic aqueductal stenosis.* **Childs.Nerv.Syst. 7:** 386-390, 1991

63. Harders, A., Kakarieka, A., and Braakman, R.: *Traumatic subarachnoid hemorrhage and its treatment with nimodipine. German tSAH Study Group [see comments].* **J.Neurosurg. 85:** 82-89, 1996

64. Hasan, D., van Peski, J., Loeve, I., Krenning, E. P., and Vermeulen, M.: *Single photon emission computed tomography in patients with acute hydrocephalus or with cerebral ischaemia after subarachnoid haemorrhage.* **J.Neurol Neurosurg.Psychiatry 54:** 490-493, 1991

65. Hawkins, D., Bowers, T. M., Bannister, C. M., and Miyan, J. A.: *The functional outcome of shunting H-Tx rat pups at different ages.* **Eur.J.Pediatr.Surg. 7 Suppl 1:** 31-34, 1997

66. Hayden, P and Shurtleff, D: *Medical management of hydrocephalus.* **Dev Med Child Neurol 14 (suppl 27):** 52-58, 1972

67. Heiss, W. D.: *Flow thresholds of functional and morphological damage of brain tissue.* **Stroke 14:** 329-331, 1983

68. Henderson, J. T., Seniuk, N. A., Richardson, P. M., Gauldie, J., and Roder, J. C.: *Systemic administration of ciliary neurotrophic factor induces cachexia in rodents.* **J.Clin.Invest. 93:** 2632-2638, 1994

69. Higashi, K., Asahisa, H., Ueda, N., Kobayashi, K., Hara, K., and Noda, Y.: *Cerebral blood flow and metabolism in experimental hydrocephalus.* **Neurol Res 8:** 169-176, 1986

70. Hirayama, A.: *Histopathological study of congenital and acquired experimental hydrocephalus.* **Brain Dev. 2:** 171-189, 1980

71. Hochwald, G. M.: *Animal models of hydrocephalus: recent developments.* **Proc.Soc.Exp.Biol.Med. 178:** 1-11, 1985

72. Hochwald, G. M., Sahar, A., Sadik, A. R., and Ransohoff, J.: *Cerebrospinal fluid production and histological observations in animals with experimental obstructive hydrocephalus.* **Exp.Neurol. 25:** 190-199, 1969

73. Hong, S. C., Goto, Y., Lanzino, G., Soleau, S., Kassell, N. F., and Lee, K. S.: *Neuroprotection with a calpain inhibitor in a model of focal cerebral ischemia.* **Stroke 25:** 663-669, 1994

74. Huttenlocher, PR: *Treatment of hydrocephalus with acetazolamide.* **J Pediatr 66:** 1023-1030, 1965

75. Ip, N. Y. and Yancopoulos, G. D.: **Ciliary neurotrophic factor and its receptor complex.* ***Prog.Growth Factor.Res 4:** 139-155, 1992

76. James-AE, Jr, Flor, W. J., Novak, G. R., Strecker, E. P., Burns, B., and Epstein, M.: *Experimental hydrocephalus.* **Exp.Eye Res. 25 Suppl:** 435-459, 1977

77. Johnson, G. V., Litersky, J. M., and Jope, R. S.: *Degradation of microtubule-associated protein 2 and brain spectrin by calpain: a comparative study.* **J.Neurochem. 56:** 1630-1638, 1991

78. Jonas, S. and Brown, J.: *Neurogenic bladder in normal pressure hydrocephalus.* **Urology. 5:** 44-50, 1975

79. Jones, H. C., Richards, H. K., Bucknall, R. M., and Pickard, J. D.: *Local cerebral blood flow in rats with congenital hydrocephalus.* **J.Cereb.Blood Flow Metab. 13:** 531-534, 1993

80. Jones, H. C., Rivera, K. M., and Harris, N. G.: *Learning deficits in congenitally hydrocephalic rats and prevention by early shunt treatment.* **Childs.Nerv.Syst. 11:** 655-660, 1995

81. Kraemer, P. J., Brown, R. W., Baldwin, S. A., and Scheff, S. W.: *Validation of a single-day Morris Water Maze procedure used to assess cognitive deficits associated with brain damage.* **Brain Res.Bull. 39:** 17-22, 1996

82. Kriebel, R. M., Shah, A. B., and McAllister, J. P.: *The microstructure of cortical neuropil before and after decompression in experimental infantile hydrocephalus.* **Exp.Neurol. 119:** 89-98, 1993

83. Kumon, Y., Sakaki, S., Watanabe, H., Nakano, K., Ohta, S., Matsuda, S., Yoshimura, H., and Sakanaka, M.: *Ciliary neurotrophic factor attenuates spatial cognition impairment, cortical infarction and thalamic degeneration in spontaneously hypertensive rats with focal cerebral ischemia.* **Neurosci.Lett. 206:** 141-144, 15-3-1996

84. Langley, M. S. and Sorkin, E. M.: *Nimodipine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in cerebrovascular disease.* **Drugs 37:** 669-699, 1989

85. LaVail, M. W., Yasumura, D., Matthes, M. T., Lau, Villacorta C., Unoki, K., Sung, C. H., and Steinberg, R. H.: *Protection of mouse photoreceptors by survival factors in retinal degenerations* . **Invest.Ophthalmol.Vis.Sci. 39:** 592-602, 1998

86. Lin, Z., Harris, C., and Lipscombe, D.: *The molecular identity of Ca channel alpha 1-subunits expressed in rat sympathetic neurons.* **J.Mol.Neurosci. 7:** 257-267, 1996

87. Litersky, J. M. and Johnson, G. V.: *Phosphorylation by cAMP-dependent protein kinase inhibits the degradation of tau by calpain.* **J.Biol.Chem. 267:** 1563-1568, 25-1-1992

88. Longatti, P. L., Canova, G., Guida, F., Carniato, A., Moro, M., and Carteri, A.: *The CSF myelin basic protein: a reliable marker of actual cerebral damage in hydrocephalus.* **J.Neurosurg.Sci. 37:** 87-90, 1993

89. Lorber, J.: *Isosorbide in the management of infantile hydrocephalus.* **Dev Med Child Neurol 25:** 502-511, 1983

90. Malashkhia, V. Y., Nadareishvili, Z. G., and Malashkhia, Y. A.: *Add-on therapy with nimodipine in intractable epilepsy of childhood.* **J Child Neurol 11:** 500-501, 1996

91. Massicotte, EM, Buist, R., and Del Bigio, M. R.: *Altered diffusion and perfusion in hydrocephalic rat brain: a magnetic resonance imaging analysis.* **J.Neurosurg. 92:** 442-447, 2000

92. Massion, P. P., Linden, A., Inoue, H., Mathy, M., Grattan, K. M., and Nadel, J. A.: *Dimethyl sulfoxide decreases interleukin-8-mediated neutrophil recruitment in the airways.* **Am.J.Physiol. 271:** L838-L843, 1996

93. Matsumae, M., Sogabe, T., Miura, I., and Sato, O.: *Energy metabolism in kaolin-induced hydrocephalic rat brain. Assessed by phosphorus (31P) magnetic resonance spectroscopy and the diversity of lactate-dehydrogenase and its isoenzyme patterns.* **Childs.Nerv.Syst. 6:** 392-396, 1990

94. Matsumoto, S., Hirayama, A., Yamasaki, S., Shirataki, K., and Fujiwara, K.: *Comparative study of various models of experimental hydrocephalus.* **Childs.Brain 1:** 236-242, 1975

95. Mayburg, O. Hydrocephalus. Its Symptomatology, Pathology, Pathogenesis and Treatment. Oskar Piest. 217. 1940. New York.
Ref Type: Book, Whole

96. McAllister, J. P. and Chovan, P.: *Neonatal hydrocephalus. Mechanisms and consequences.* **Neurosurg.Clin.N.Am. 9:** 73-93, 1998

97. McAllister, J. P. and Chovan, P.: *Neonatal hydrocephalus. Mechanisms and consequences.* **Neurosurg.Clin.N.Am. 9:** 73-93, 1998

98. McGraw, C. P.: *Treatment of cerebral infarction with dimethyl sulfoxide in the mongolian gerbil.* **Ann.N.Y.Acad.Sci. 411:** 278-285, 1983

99. McLone, D. G., Bondareff, W., and Raimondi, A. J.: *Hydrocephalus-3, a Murine mutant. II. Changes in the brain extracellular space.* **Surg.Neurol. 1:** 233-242, 1973

100. Mee, E., Dorrance, D., Lowe, D., and Neil, Dwyer G.: *Controlled study of nimodipine in aneurysm patients treated early after subarachnoid hemorrhage.* **Neurosurgery 22:** 484-491, 1988

101. Melloni, E. and Pontremoli, S.: *The calpains.* **Trends.Neurosci. 12:** 438-444, 1989

102. Messert, B. and Baker, N. H.: *Syndrome of progressive spastic ataxia and apraxia associated with occult hydrocephalus.* **Neurology 16:** 440-452, 1966

103. Milhorat, T. H.: Hydrocephalus: Pathophysiology and Clinical Features

104. Milhorat, T. H., Clark, R. G., Hammock, M. K., and McGrath, P. P.: *Structural, ultrastructural, and permeability changes in the ependyma and surrounding brain favoring equilibration in progressive hydrocephalus.* **Arch.Neurol. 22:** 397-407, 1970

105. Miyagami, M., Nakamura, S., Mukakami, T., Koga, N., and Moriyasu, N.: *Electron microscopic study of ventricular wall and choroid plexus in experimentally-induced hydrocephalic dogs.* **Neurol.Med.Chir.Tokyo. 16:** 15-21, 1976

106. Miyazawa, T. and Sato, K.: *Learning disability and impairment of synaptogenesis in HTX-rats with arrested shunt-dependent hydrocephalus.* **Childs.Nerv.Syst. 7:** 121-128, 1991

107. Neil, Dwyer G., Mee, E., Dorrance, D., and Lowe, D.: *Early intervention with nimodipine in subarachnoid haemorrhage.* **Eur.Heart J. 8 Suppl K:** 41-47, 1987

108. Newcomb, J. K., Kampfl, A., Posmantur, R. M., Zhao, X., Pike, B. R., Liu, S. J., Clifton, G. L., and Hayes, R. L.: *Immunohistochemical study of calpain-mediated breakdown products to alpha-spectrin following controlled cortical impact injury in the rat.* **J.Neurotrauma. 14:** 369-383, 1997

109. Nicholson, C., Bruggencate, G. T., Steinberg, R., and Stockle, H.: *Calcium modulation in brain extracellular microenvironment demonstrated with ion-selective micropipette.* **Proc.Natl.Acad.Sci.U.S.A. 74:** 1287-1290, 1977

110. Ochs, S., Pourmand, R., Jersild, R. A., and Friedman, R. N.: *The Origin and Nature of Beading: A Reversible Transformation of the Shape of Nerve Fibers.* **Progress in Neurobiology 52:** 391-426, 1997

111. Oi, S., Ijichi, A., and Matsumoto, S.: *Immunohistochemical evaluation of neuronal maturation in untreated fetal hydrocephalus.* **Neurol.Med.Chir.Tokyo. 29:** 989-994, 1989

112. Oi, S., Yamada, H., Sato, O., and Matsumoto, S.: *Experimental models of congenital hydrocephalus and comparable clinical problems in the fetal and neonatal periods [see comments].* **Childs.Nerv.Syst. 12:** 292-302, 1996

113. Oorschot, D. E. and Jones, D. G.: *Axonal regeneration in the mammalian central nervous system. A critique of hypotheses.* **Adv.Anat.Embryol.Cell Biol. 119:** 1-118, 1990

114. Page, R. B.: *Scanning electron microscopy of the ventricular system in normal and hydrocephalic rabbits. Preliminary report and atlas.* **J.Neurosurg. 42:** 646-664, 1975

115. Page, R. B., Rosenstein, J. M., Dovey, B. J., and Leure, duPree AE: *Ependymal changes in experimental hydrocephalus.* **Anat.Rec. 194:** 83-103, 1979

116. Pelliccia, A., Sciaretta, A., and Matricardi, M.: *Nimodipine treatment for drug-resistant childhood epilepsy [letter].* **Dev.Med.Child Neurol. 32:** 1114-1990

117. Penn, R. D. and Bacus, J. W.: *The brain as a sponge: a computed tomographic look at Hakim's hypothesis.* **Neurosurgery 14:** 670-675, 1984

118. Petruk, K. C., West, M., Mohr, G., Weir, B. K., Benoit, B. G., Gentili, F., Disney, L. B., Khan, M. I., Grace, M., Holness, R. O., and et, al: *Nimodipine treatment in poor-grade aneurysm patients. Results of a multicenter double-blind placebo-controlled trial.* **J.Neurosurg. 68:** 505-517, 1988

119. Pickard, J. D., Murray, G. D., Illingworth, R., Shaw, M. D., Teasdale, G. M., Foy, P. M., Humphrey, P. R., Lang, D. A., Nelson, R., Richards, P., and et, al: *Effect of oral nimodipine on cerebral infarction and outcome after subarachnoid haemorrhage: British aneurysm nimodipine trial.* **BMJ. 298:** 636-642, 11-3-1989

120. Poduslo, J. F. and Curran, G. L.: *Permeability at the blood-brain and blood-nerve barriers of the neurotrophic factors: NGF, CNTF, NT-3, BDNF.* **Brain Res.Mol.Brain Res. 36:** 280-286, 1996

121. Povlishock, J. T. and Becker, D. P.: *Fate of reactive axonal swellings induced by head injury.* **Lab.Invest. 52:** 540-552, 1985

122. Pudenz, R. H, Russell, F. E, Hurd, A. H., and Sheldon, C. H.: *Ventriculoauriculostomy A technique for shunting CSF.* **J.Neurosurg. 14:** 171-179, 1957

123. Raimondi, A. J., Bailey, O. T., McLone, D. G., Lawson, R. F., and Echeverry, A.: *The pathophysiology and morphology of murine hydrocephalus in Hy-3 and Ch mutants.* **Surg.Neurol. 1:** 50-55, 1973

124. Richardson, P. M.: *Ciliary neurotrophic factor: a review.* **Pharmacol.Ther. 63:** 187-198, 1994

125. Rowlatt, U.: *The microscopic effects of ventricular dilatation without increase in head size*. **J.Neurosurg. 48:** 957-961, 1978

126. Rubin, R. C., Hochwald, G., Tiell, M., Liwnicz, B., and Epstein, F.: *Reconstitution of the cerebral cortical mantle in shunt-corrected hydrocephalus*. **Dev.Med.Child Neurol.Suppl.** 151-156, 1975

127. Rubin, R. C., Hochwald, G. M., Tiell, M., Mizutani, H., and Ghatak, N.: *Hydrocephalus: I. Histological and ultrastructural changes in the pre-shunted cortical mantle*. **Surg.Neurol. 5:** 109-114, 1976

128. Russell, D. S.: *Observations on the pathology of hydrocephalus*. **Med Res Counc (GB) Spec Rep Ser 265:** 1-138, 1949

129. Saeed, S. A., Karimi, S. J., and Suria, A.: *Differential effects of dimethyl sulfoxide on human platelet aggregation and arachidonic acid metabolism*. **Biochem Med Metab.Biol. 40:** 143-150, 1988

130. Sattler, R., Tymianski, M., Feyaz, I., Hafner, M., and Tator, C. H.: *Voltage-sensitive calcium channels mediate calcium entry into cultured mammalian sympathetic neurons following neurite transection*. **Brain Res. 719:** 239-246, 6-5-1996

131. Schlaepfer, W. W. and Bunge, R. P.: *Effects of calcium ion concentration on the degeneration of amputated axons in tissue culture*. **J.Cell Biol. 59:** 456-470, 1973

132. Scriabine, A. and van-den-Kerckhoff, W.: *Pharmacology of nimodipine. A review*. **Ann.N.Y.Acad.Sci. 522:** 698-706, 1988

133. Sendtner, M., Kreutzberg, G. W., and Thoenen, H.: *Ciliary neurotrophic factor prevents the degeneration of motor neurons after axotomy*. **Nature 345:** 440-441, 31-5-1990

134. Shahar, E., Sagy, M., Koren, G., and Barzilay, Z.: *Calcium blocking agents in pediatric emergency care*. **Pediatr.Emerg.Care 6:** 52-57, 1990

135. Shih, W. J. and Tasdemiroglu, E.: *Reversible hypoperfusion of the cerebral cortex in normal-pressure hydrocephalus on technetium-99m-HMPAO brain SPECT images after shunt operation*. **J.Nucl.Med 36:** 470-473, 1995

136. Shinnar, S and Gammon, K: *Management of hydrocephalus in infancy: use of acetazolamide and furosemide to avoid cerebrospinal fluid shunt.* **J Pediatr 107:** 31-37, 1985

137. Silver, I. A. and Erecinska, M.: *Intracellular and extracellular changes of [Ca2~] in hypoxia and ischemia in rat brain in vivo.* **J.Gen.Physiol. 95:** 837-866, 1990

138. Simon, R. P., Griffiths, T., Evans, M. C., Swan, J. H., and Meldrum, B. S.: *Calcium overload in selectively vulnerable neurons of the hippocampus during and after ischemia: an electron microscopy study in the rat.* **J.Cereb.Blood Flow Metab. 4:** 350-361, 1984

139. Sinson, G., Perri, B. R., Trojanowski, J. Q., Flamm, E. S., and McIntosh, T. K.: *Improvement of cognitive deficits and decreased cholinergic neuronal cell loss and apoptotic cell death following neurotrophin infusion after experimental traumatic brain injury.* **J.Neurosurg. 86:** 511-518, 1997

140. Smith, D. H., Okiyama, K., Thomas, M. J., Claussen, B., and McIntosh, T. K.: *Evaluation of memory dysfunction following experimental brain injury using the Morris water maze.* **J.Neurotrauma. 8:** 259-269, 1991

141. Sogabe, T., Matsumae, M., Sato, O., and Miura, I.: *Change in glucose metabolism with time in hydrocephalic rats.* **Biochem Int. 19:** 513-518, 1989

142. Sutton, L. N., Wood, J. H., Brooks, B. R., Barrer, S. J., Kline, M., and Cohen, S. R.: *Cerebrospinal fluid myelin basic protein in hydrocephalus.* **J.Neurosurg. 59:** 467-470, 1983

143. Tan, S. A., Deglon, N., Zurn, A. D., Baetge, E. E., Bamber, B., Kato, A. C., and Aebischer, P.: *Rescue of motoneurons from axotomy-induced cell death by polymer encapsulated cells genetically engineered to release CNTF.* **Cell Transplant. 5:** 577-587, 1996

144. Thome, J., Nara, K., Foley, P., Michel, T., Gsell, W., Retz, W., Rosler, M., and Riederer, P.: *Ciliary neurotrophic factor (CNTF) genotypes: influence on choline acetyltransferase (ChAT) and acetylcholine esterase (AChE) activities and neurotrophin 3 (NT3) concentration in human post mortem brain tissue.* **J.Hirnforsch. 38:** 443-451, 1997

145. Thompson, N. M., Fletcher, J. M., Chapieski, L., Landry, S. H., Miner, M. E., and Bixby, J.: *Cognitive and motor abilities in preschool hydrocephalics.* **J.Clin.Exp.Neuropsychol. 13:** 245-258, 1991

146. Torvik, A., Stenwig, A. E., and Finseth, I.: *The pathology of experimental obstructive hydrocephalus. A scanning electron microscopic study.* **Acta Neuropathol.Berl. 54:** 143-147, 1981

147. Tymianski, M. and Tator, C. H.: *Normal and abnormal calcium homeostasis in neurons: a basis for the pathophysiology of traumatic and ischemic central nervous system injury.* **Neurosurgery 38:** 1176-1195, 1996

148. Uematsu, D., Araki, N., Greenberg, J. H., and Reivich, M.: *Alterations in cytosolic free calcium in the cat cortex during bicuculline-induced epilepsy.* **Brain Res.Bull. 24:** 285-288, 1990

149. Waldemar, G., Schmidt, J. F., Delecluse, F., Andersen, A. R., Gjerris, F., and Paulson, O. B.: *High resolution SPECT with [99mTc]-d,l-HMPAO in normal pressure hydrocephalus before and after shunt operation.* **J.Neurol Neurosurg.Psychiatry 56:** 655-664, 1993

150. Wang, W., Zhao, F., Yu, X. H., Lin, G. Z., Xu, Z. K., Dong, S. M., Dong, X. F., Wang, S. Y., and Liu, L. S.: *Effect of treatment with nimodipine in patients with mild and moderate essential hypertension.* **J.Hum.Hypertens. 4:** 149-150, 1990

151. Weller, R. O., Mitchell, J., Griffin, R. L., and Gardner, M. J.: *The effects of hydrocephalus upon the developing brain. Histological and quantitative studies of the ependyma and subependyma in hydrocephalic rats.* **J.Neurol.Sci. 36:** 383-402, 1978

152. Weller, R. O. and Shulman, K.: *Infantile hydrocephalus: clinical, histological, and ultrastructural study of brain damage.* **J.Neurosurg. 36:** 255-265, 1972

153. Weller, R. O. and Williams, B. N.: *Cerebral biopsy and assessment of brain damage in hydrocephalus.* **Arch.Dis.Child 50:** 763-768, 1975

154. Weller, R. O., Wisniewski, H., Shulman, K., and Terry, R. D.: *Experimental hydrocephalus in young dogs: histological and ultrastructural study of the brain tissue damage.* **J.Neuropathol.Exp.Neurol. 30:** 613-626, 1971

155. Westenbroek, R. E., Bausch, S. B., Lin, R. C., Franck, J. E., Noebels, J. L., and Catterall, W. A.: - *Upregulation of L-type Ca2- channels in reactive astrocytes after brain injury, hypomyelination, and ischemia.* - **J Neurosci 1998 Apr 1;18(7):2321-34** 2321-2344,

156. Whittall, K. P., MacKay, A. L., Graeb, D. A., Nugent, R. A., Li, D. K., and Paty, D. W.: *In vivo measurement of T2 distributions and water contents in normal human brain.* **Magn.Reson.Med. 37:** 34-43, 1997

157. Windisch, M., Gschanes, A., and Hutter, Paier B.: *Neurotrophic activities and therapeutic experience with a brain derived peptide preparation.* **J.Neural Transm.Suppl. 53:** 289-298, 1998

158. Wiswell, T. E., Tuttle, D. J., Northam, R. S., and Simonds, G. R.: *Major congenital neurologic malformations. A 17-year survey [see comments].* **Am.J.Dis.Child 144:** 61-67, 1990

159. Woodruff, R. H. and Franklin, R. J.: *Growth factors and remyelination in the CNS.* **Histol.Histopathol. 12:** 459-466, 1997

160. Wu, P., Price, P., Du, B., Hatch, W. C., and Terwilliger, E. F.: *Direct cytotoxicity of HIV-1 envelope protein gp120 on human NT neurons.* **Neuroreport. 7:** 1045-1049, 10-4-1996

161. Xu, B., Dube, M. G., Kalra, P. S., Farmerie, W. G., Kaibara, A., Moldawer, L. L., Martin, D., and Kalra, S. P.: *Anorectic effects of the cytokine, ciliary neurotropic factor, are mediated by hypothalamic neuropeptide Y: comparison with leptin.* **Endocrinology 139:** 466-473, 1998

162. Yakovlev, P. I.: *Paraplegia of hydrocephalus. A clinical note and interpretation.* **Am.J.Ment.Defic. 51:** 561-576, 1947

163. Yamada, H., Yokota, A., Furuta, A., and Horie, A.: *Reconstitution of shunted mantle in experimental hydrocephalus.* **J.Neurosurg. 76:** 856-862, 1992

164. Zhang, Y. W. and Del Bigio, M. R.: *Growth-associated protein-43 is increased in cerebrum of immature rats following induction of hydrocephalus.* **Neuroscience 86:** 847-854, 1998