Early Functional-Structural Repair of Macaque Strabismus

Tychsen, Lawrence

Ophthalmology & Visual Sciences

Tel: 314-362-3743 Fax: 314-362-3131

School of Medicine

4. Human Subjects

5. Vertebrate Animals

6. Dates of Proposed Period of Support

7. Costs Requested for Initial Budget Period

8. Costs Requested for Proposed Period of Support

9. Applicant Organization

10. Type of Organization

11. Entity Identification Number

12. Administrative Official to be Notified if Award is Made

13. Official Signing for Applicant Organization

14. Principal Investigator/Program Director Assurance: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application.

15. Applicant Organization Certification and Acceptance: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application.

Washington University

Ophthalmology & Visual Sciences

Campus Box 8096

St. Louis, Missouri 63110

E-mail: ggcinfo@msnotes.wustl.edu

Department of Health and Human Services

Public Health Services

MAR 802002

Application

Do not exceed 20 pages unless restrictions, including space for calculations, require additional pages.

**PI: TYCHSEN, LAWRENCE**

Council: 10/2002

2 R01 EY010214-08

IPF: 9083901

Received: 03/01/2002

**Address:** 660 South Euclid Avenue

St. Louis, Missouri 63110

**Telephone:** 314-362-6150

**Fax:** 314-362-0315

**E-mail:** ggcinfo@msnotes.wustl.edu

**Institutional Profile File Number:** (if known) 06-855-2207

**Congressional District:** 01

**DUNS No. (if available):**

**Public:** → X Federal

Local

**Private:** → Private Nonprofit

**For-profit:** → General

Small Business

**Woman-owned:**

**Socially and Economically Disadvantaged:**

**Signature of PI/PD Named in 3a.**

(Im ink. "Per" signature not acceptable.)

**Signature of Official Named in 13.**

(Im ink. "Per" signature not acceptable.)

**Date:** 2/26/02

**Date:** 12/28/02

**Total Costs ($):** 2,811,229

**Direct Costs ($):** 2,810,810

**Total Costs ($):** 561,282

**Direct Costs ($):** 422,550

**Total Costs ($):** 2,015,810

**Direct Costs ($):** 282,105

**Total Costs ($):** 2,811,229

**Direct Costs ($):** 0

**Total Costs ($):** 0

**Per" signature not acceptable.)

**Signature of Official Named in 13.**

(Im ink. "Per" signature not acceptable.)

**Date:** 12/28/02
DESCRIPTION: State the application's broad, long-term objectives and specific aims, making reference to the health relatedness of the project. Describe concisely the research design and methods for achieving these goals. Avoid summaries of past accomplishments and the use of the first person. This abstract is meant to serve as a succinct and accurate description of the proposed work when separated from the application. If the application is funded, this description, as is, will become public information. Therefore, do not include proprietary/confidential information. DO NOT EXCEED THE SPACE PROVIDED.

The long-term goal of this research is to reveal the neural mechanisms that explain the behavioral deficits of infantile strabismus. The findings of our progress report indicate that infant monkeys with strabismus are an appropriate behavioral model of the human disorder, and have structural defects of visual cortex that help explain the behaviors. The specific goal of this research is to determine the age at which treatment of strabismus in macaques is capable of repairing these behavioral and structural deficits. The following hypotheses will be tested in each animal: Hypothesis 1. Macaques with strabismus-onset at birth have maldeveloped pursuit eye movements that can be repaired if the strabismus is corrected by age 6-9 weeks. Newborn macaques will be reared wearing prism-goggles to cause optical strabismus. The strabismus will be repaired by removing the prisms at one of four postnatal ages (3, 6, 9 or 12 wks), corresponding to "early"(3 mos human), "early-intermediate"(6 mos), "delayed-intermediate" (9 mos) and "delayed"(12 mos) strabismus surgery in human infants. Pursuit will then be recorded in each macaque. If pursuit is more normal in monkeys realigned at earlier ages, early realignment will be considered to be effective for repairing pursuit pathways. Hypothesis 2: Macaques with strabismus-onset 3 weeks after birth have maldeveloped pursuit; the maldevelopment can be repaired if the duration of strabismus does not exceed 3 weeks. Optical strabismus will be induced after 3 wks (3 mos human) of normal visual experience, and will endure for 3, 6, 9 or 12 wks. After these intervals the strabismus will be repaired by prism removal and pursuit will be recorded. If pursuit is more normal in monkeys realigned after shorter durations of strabismus, prompt realignment will be considered to be effective for repairing pursuit. Hypothesis 3: Strabismic macaques have maldeveloped short-latency ocular following and vergence eye movements that can be repaired if the strabismus is corrected within the time-frames specified in Hypotheses 1 and 2. Ocular following and vergence eye movements will be recorded in monkeys with optical strabismus whose eyes are realigned at the post-natal ages designated under Hypotheses 1 and 2. If the amplitude and velocity of these movements are significantly greater in monkeys realigned at earlier ages/shorter durations, early/prompt realignment will be considered to be effective for repairing ocular following and short-latency vergence pathways. Hypothesis 4: Strabismic macaques have structural abnormalities in visual cortex that: a) correlate with their pursuit, ocular following and vergence behavioral "indicators", and b) can be repaired by early realignment of the eyes; the earlier the realignment, the greater the neuroanatomic recovery. Ocular dominance columns (ODCs) of V1 will be labeled by transneuronal transport of [3H]proline, and BDA will be injected into ODCs to label binocular connections (horizontal projections). If the number of binocular connections increases in animals realigned at earlier ages/shorter durations, early/prompt realignment will be considered to be effective for repairing binocular cortical circuits that mediate pursuit, disparity vergence and ocular following. The experiments proposed here could lead to refined treatments and functional cures in humans, and reveal the biological mechanisms responsible for this common childhood visual disorder.

PERFORMANCE SITE(S) (organization, city, state)

Washington University, St. Louis, Missouri

KEY PERSONNEL. See instructions. Use continuation pages as needed to provide the required information in the format shown below. Start with Principal Investigator. List all other key personnel in alphabetical order, last name first.

Name: Lawrence Tychsen
Organization: Washington University
Role on Project: Principal Investigator

Disclosure Permission Statement. Applicable to SBIR/STTR Only. See instructions. □ Yes □ No

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Number pages consecutively at the bottom throughout the application. No not use suffixes such as 3a, 3b.
RESEARCH GRANT

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<td>Biographical Sketch—Principal Investigator/Program Director (Not to exceed four pages)</td>
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<td>Other Biographical Sketches (Not to exceed four pages for each)</td>
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<td>Resources</td>
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Research Plan

Introduction to Revised Application (Not to exceed 3 pages) ................................................. N/A
Introduction to Supplemental Application (Not to exceed one page) ................................................ N/A

A. Specific Aims ........................................................................................................................................ 27
B. Background and Significance ........................................................................................................... 28
C. Preliminary Studies/Progress Report/ Phase I Progress Report (SBIR/STTR Phase II ONLY) .......... 31
D. Research Design and Methods .......................................................................................................... 38
E. Human Subjects .................................................................................................................................... N/A
   Protection of Human Subjects (Required if Item 4 on the Face Page is marked “Yes”) .............. N/A
   Inclusion of Women (Required if Item 4 on the Face Page is marked “Yes”) ............................. N/A
   Inclusion of Minorities (Required if Item 4 on the Face Page is marked “Yes”) ..................... N/A
   Inclusion of Children (Required if Item 4 on the Face Page is marked “Yes”) ........................ N/A
   Data and Safety Monitoring Plan (Required if Item 4 on the Face Page is marked “Yes” and a Phase I, II, or III clinical trial is proposed) ............................................................ N/A
F. Vertebrate Animals ........................................................................................................................... 50
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I. Consultants ........................................................................................................................................... N/A
J. Product Development Plan (SBIR/STTR Phase II and Fast-Track ONLY) ......................................... N/A

Checklist .................................................................................................................................................... 61

* SBIR/STTR Phase I applications: Items A-D of the Research Plan are limited to 15 pages.

Appendix (Five collated sets. No page numbering necessary for Appendix.)

Appendices NOT PERMITTED for Phase I SBIR/STTR unless specifically solicited.

Number of publications and manuscripts accepted for publication (not to exceed 10)
Other items (list): N/A
### DETAILED BUDGET FOR INITIAL BUDGET PERIOD

**DIRECT COSTS ONLY**

**PERSONNEL** *(Applicant organization only)*

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**SUBTOTALS**

|               |                 |                     |                   |                   | 134,413         | 25,779         | 160,192 |

**CONSULTANT COSTS**

**EQUIPMENT** *(Itemize)*

**SUPPLIES** *(Itemize by category)*

- Computer & Software: 6,960
- Computer Supplies: 2,875
- Histology Chemicals & Glassware: 625
- Photographic Supplies: 1,000
- Electrical Components: 2,500

**TRAVEL**

- PI & Staff Scientist to ARVO & Society for Neuroscience Meetings & Yerkes: 4,000

**PATIENT CARE COSTS**

- INPATIENT
- OUTPATIENT

**ALTERATIONS AND RENOVATIONS** *(Itemize by category)*

- Other Expenses: 58,606

**OTHER EXPENSES** *(Itemize by category)*

- Monkey per diem (see justification): 22,787
- Long Distance Telephone (Yerkes): 250
- Equipment maintenance & repair: 3,000
- Upgrade Coil Box: 13,569
- Publication Costs: 2,000
- Computer Programming: 17,000

**SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD**

|               |                 |                     |                   |                   | 236,758         |               |

**CONSORTIUM/CONTRACTUAL**

**DIRECT COSTS**

|               |                 |                     |                   |                   | 116,120         |               |

**FACILITIES AND ADMINISTRATION COSTS**

|               |                 |                     |                   |                   | 69,672          |               |

**TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD** *(Item 7a, Face Page)*

|               |                 |                     |                   |                   | 422,550         |               |

**SBIR/STTR Only: FIXED FEE REQUESTED**
## BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
### DIRECT COSTS ONLY

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**TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD (Item 8a, Face Page)** $2,105,810

SBIR/STTR Only

**Fixed Fee Requested**

SBIR/STTR Only: Total Fixed Fee Requested for Entire Proposed Phase II Period
(Add Total Fixed Fee amount to "Total direct costs for entire proposed project period" above and Total F&A indirect costs from Checklist Form Page, and enter these as "Costs Requested for Proposed Period of Support on Face Page, Item 8b."

$0

**JUSTIFICATION.** Follow the budget justification instructions exactly. Use continuation pages as needed.

All categories have been increased 4% per annum.
Personnel

Lawrence Tychsen, the principal investigator, will be responsible for all aspects of the research project, including the design of experiments, the organization of the laboratory facility, the conduct of the research and animal surgery, the analysis of behavioral and anatomic data, and the presentation of results and conclusions in papers and reports. The PI has experience in eye movement and VEP recording in awake primates and, through collaboration with [redacted] over the last 10 years, has learned the neuroanatomic techniques necessary to perform the proposed research. The PI will devote [redacted] of his time to the research outlined in this proposal for the 5-year period extending from 12/01/02 through 11/30/07.

[redacted] the Co-investigator, will participate on all neuroanatomic aspects of this study during the five years of the proposed research. He is an expert in studies of monkey and human visual cortex using neuroanatomic labeling techniques, and has collaborated closely with the principal investigator in obtaining the neuroanatomic data of the Progress Report. [redacted] effort to this project.

[redacted] the Staff Scientist, will assist the principal investigator in animal training and in daily conduct of the eye movement experiments. He is versed in physiological recordings from alert animals and in operation of the computers and associated electronics used for data collection and analysis. He is needed to oversee daily animal care, to administer medications to the animals, to obtain weights and monitor water deprivation schedules, to chair and transport the animals each day to and from the laboratory, to clean and sterilize supplies for animal surgeries, to help stock supplies and clean equipment, and to assist the P.I. at animal surgeries. [redacted] support is requested for the Staff Scientist.

[redacted] the Histology Technician, will assist the principal and co-investigator in histological processing and mounting of visual cortex tissue, in performing histologic measurements, in photomicrography, and in statistical analysis of anatomic results. She is trained in operation of the microscopes, camera lucida devices, and imaging software needed for analyzing anatomic results in the proposed research. [redacted] support is requested for the histology technician.

Supplies:

Pentium based PC ($2,000) - We will replace the Macintosh which controls the eye movement experiments and collects the eye movement data. The Mac is eight years old and is networked to a PC; the networking was required to generate the random dot binocular disparity display. By upgrading we will be able to (a) consolidate stimulus display and recording in a single PC and (b) simplify the electronic control of experiments by eliminating an external eye-on-target detector circuit we have been using. The new PC and software code will provide more seamless monitoring and display of binocular eye-target position during experiments and allow replay during analysis.

Monitor ($1,460) - An 18 in. high resolution LCD flat panel monitor is needed for the Pentium based PC to display binocular eye position and target position tracings in real-time during experiments.

Computer software ($3,500) - Software requests (Spike 2 version 4 & PCI interface for 1401 plus) are for updating of programs currently used for stimulus generation, eye movement recording and analysis (Spike 2 version 4 & PCI interface for 1401 plus), neuroanatomic analysis, statistical analysis, preparation of graphs and word processing.

The total cost for the computer, monitor and software requested above is $6,960.
Computer supplies ($2,875) - We will use approximately 20 Jaz drive cartridges at $100 each to store the large amount of digital raw data that will accumulate in these experiments. Five 20 GB Peerless disks at $175 each are required to store analyzed data. Costs for laser printer supplies, replacement boards, cables and mouses are included.

Histology chemicals and glassware ($625) - These supply costs represent the best estimate of expenditures expected in year 01. We will need to buy reagents for cytochrome oxidase histochemistry and general purpose chemicals as well as the BDA and tritiated proline tracer. The budget for glassware contains microscopic slides, coverslips, slide boxes, large staining dishes and staining racks, and glass tubing for making injection pipettes.

Photographic supplies ($1,000) - These include paper, chemicals, film and processing of film.

Electrical components ($2,500) - These include the wire needed to fashion eye coils, the coil connectors, cables on the primate chair that inevitably break with manipulation by the monkeys; parts for electronic servos and interfaces we use for animal training; cables to/from the experimental chamber to the amplifiers/computers; lasers, LEDs and small parts used for the eye movement experiment; and small tools for repairs to the chair.

Travel

A modest amount ($4,000) is requested for the Principal Investigator and Staff Scientist to travel to the annual meetings of the Association for Research in Vision and Ophthalmology and the Society for Neuroscience. The PI or Staff Scientist will travel twice per year to Yerkes for on site consultations with the sub-contractor, Dr. [Name Redacted] who provides daily supervision of the nursery prism-rearing.

Other Expenses

Animals- 32 infant monkeys will be purchased from Yerkes over a period of 5 years as described under Contractual Costs. The monkeys will be transferred to Washington University. Animal care for the monkeys is as follows:

01 year 5 monkeys x 243 days per monkey x $8.135 per day and 13 monkeys x 122 days per monkey x $8.135 per day; 02 year 21 monkeys x 122 days per monkey x $8.135 per day and 13 monkeys x 243 days per monkey x $8.135 per day; 03 year 21 monkeys x 212 days per monkey x $8.135 per day and 15 monkeys x 61 days per monkey x $8.135 per day; 04 year 21 monkeys x 212 days per monkey x $8.135 per day, 18 monkeys x 92 days per monkey x $8.135 per day and 15 monkeys x 61 days per monkey x $8.135 per day; 05 year 13 monkeys x 122 days per monkey x $8.135 per day, 18 monkeys x 92 days per monkey x $8.135 per day, 21 monkeys x 90 days per monkey x $8.135 per day and 15 monkeys x 61 days per monkey x $8.135 per day.

Operating room expenses - The cost of the anesthetics, antibiotics, analgesics, intravenous fluids, tubes, anesthesia tech, disposable gowns, gloves, sutures, broken instruments are included in this amount for each surgery based on average fees incurred during the current research period. Thirty-two animals will require two major surgical procedures at Washington University during the proposed research. We anticipate 0 procedures in the year 01, 8 procedures in the year 02, 16 procedures in the year 03, 16 procedures in the year 04 and 24 procedures in year 05.
Equipment maintenance and repair ($3,000) - Training and recording from alert monkeys entails high wear and tear on equipment surrounding the animals and their primate chair. On average twice per month some component of the chair or associated electronics needs repair because of the animal's destructive habits. We estimate labor needs of approximately 5 hours/month for maintenance and repair at an estimated $50/hr (this includes special materials costs).

Publication costs - We have also requested funds for publication costs @ $2,000.

Long distance telephone charges; conversations with Yerkes @ $250.

Computer programming ($17,000) - Funds are requested for a commercial programmer to modify software in the CED Spike 2, C++/Open GL and Igor programming languages used by the P.I., the Staff Scientist, and students in the laboratory for purposes of animal training, stimulus presentation, eye movement recording, analysis and graphical illustration of results. The help of the commercial programmer has proved invaluable during the current research period and he is well versed in our electronic systems and the optimal application of software to achieve our research goals. He will be rewriting and debugging an extensive volume of code in eliminating our external eye-on-target detector and consolidating the random dot stimulus delivery and eye movement recording into a single PC. We anticipate 4-5 weeks (200 hours of programming) in year 01 and 100 hours per years 02-05.

Phase Angle Search Coil System ($13,569) - The electronics of the current system are over 15 years old, have required repair several times by our electrical engineer in the last two years, and need to be upgraded to expand our recording capabilities. The cost includes on site installation of power driver upgrades, a new Dual Phase Sensitive Detector with preamplifier to record head position, and Dual Torsion Detectors to provide two channels for recording torsional eye movements.

Contractual Costs

See attached subcontract for early animal rearing work to be performed at the Yerkes Regional Primate Center.
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**Total Animal Expense**

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**Total Pet Died**

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**Juvenile Pet Died**
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**Project Year 10 (12/10/04 - 11/30/05)**

**Project Year 9 (12/1/03 - 11/30/04)**

**Project Year 8 (12/1/02 - 11/30/03)**

**TYCISSEN, Lawrence**

**Typhoon RoI EVY214-03**
### Total Housing

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<th>Jan</th>
<th>Feb</th>
<th>Mar</th>
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<th>Jun</th>
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### Juvenile Housing

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### Summary

- Total Housing: 661
- Adult Housing: 661
- Juvenile Housing: 661
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Surgical Schedule
# DETAILED BUDGET FOR INITIAL BUDGET PERIOD

## DIRECT COSTS ONLY

**FROM** 12/01/02  
**THROUGH** 11/30/07

<table>
<thead>
<tr>
<th>NAME</th>
<th>ROLE ON PROJECT</th>
<th>TYPE OF APPT. (months)</th>
<th>% EFFORT ON PROJ.</th>
<th>INST. BASE SALARY</th>
<th>SALARY REQUESTED</th>
<th>FRINGE BENEFITS</th>
<th>DOLLAR AMOUNT REQUESTED (omit cents)</th>
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<td>$17,052 $3,956 $21,008</td>
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**SUBTOTALS**  
$51,421 $11,930 $63,351

## CONSULTANT COSTS

$0

## EQUIPMENT (Itemize)

$0

## SUPPLIES (Itemize by category)

- Acquisition cost: $4,000 X 9 monkeys = $36,000; per diem (2/01/03-9/30/03) = $15,239
- Goggles/helmets/lenses: $100 X 9 monkeys = $900
- Pre-assignment Ophthalmological Assessments for 9 monkeys = $630

$52,769

## TRAVEL

$0

## PATIENT CARE COSTS

- INPATIENT  
- OUTPATIENT

$0

## ALTERATIONS AND RENOVATIONS (Itemize by category)

$0

## OTHER EXPENSES (Itemize by category)

$0

**SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD**  
$116,120

**CONSORTIUM/CONTRACTUAL COSTS**

- DIRECT COSTS
- FACILITIES AND ADMINISTRATIVE COSTS  

$69,672

**TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD**  
$185,792

**SBIR/STTR Only: FIXED FEE REQUESTED**
YERKES REGIONAL PRIMATE RESEARCH CENTER CONTRACTUAL COSTS

Principal Investigator/Program Director (Last, first, middle): TYCHSEN, Lawrence

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

<table>
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<tr>
<th>BUDGET CATEGORY</th>
<th>INITIAL BUDGET PERIOD (from Form Page 4)</th>
<th>ADDITIONAL YEARS OF SUPPORT REQUESTED</th>
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<th>4th</th>
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TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD (Item 8a, Face Page) $799,833

SBIR/STTR Only
Fixed Fee Requested

SBIR/STTR Only: Total Fixed Fee Requested for Entire Proposed Phase II Period
(Add Total Fixed Fee amount to "Total direct costs for entire proposed project period" above and Total F&A/indirect costs from Checklist Form Page, and enter these as "Costs Requested for Proposed Period of Support on Face Page, Item 8b.

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.
BUDGET JUSTIFICATION FOR YERKES REGIONAL PRIMATE RESEARCH CENTER

CONTRACTUAL COSTS

Personnel

Ph.D. Principal Investigator (effort: ...) will be responsible for the overall administration and supervision of the project at the Yerkes Regional Primate Research Center of Emory University. Activities include all aspects of the rearing of 35 infant rhesus monkeys fitted with goggles to induce eye misalignment (strabismus) via prism lenses. The goggles and helmets are manufactured at Yerkes by [ ] and custom-fitted for each monkey; as they grow, throughout the rearing period. The rearing protocol requires that either ... or her trained personnel perform goggle and lens cleanliness checks at least hourly, and conduct goggle/helmet maintenance daily, during the 9 hour work day (lights on period), 7 days per week. The actual date of assignment of each monkey may occur during any month of the birthing period, which ranges from February to July, and the monkeys will be transferred to Dr. Tychsen’s laboratory by October.

To be appointed Research Specialist (effort: ...) will assist ... in all of the laboratory activities (goggle and helmet manufacture/fitting/maintenance) associated with the prism lens rearing of the 35 monkeys requested for this project. Since the number of monkeys each year (8-9) is rather large, and the time scheduled for each routine task is short, yet occurs all day, 7 days a week, a highly-skilled full-time research assistant is required for the duration of the rearing period.

To be appointed Student Assistant/Technician, (effort: ...) will be responsible for assisting the PI in all aspects of the project. Since the rearing conditions require that the monkeys be monitored from 8:00 a.m. to 6:00 p.m., 7 days a week, this person will cover the weekend shifts, and other times when the PI and the Research Specialist are not available.

Supplies

<table>
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<tr>
<th>Item</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
<th>Year 4</th>
<th>Year 5</th>
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<tr>
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<td>9</td>
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<td>per diem (2/1 to 9/30)</td>
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<td>69,225</td>
<td>20,385</td>
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<td>Goggles/Helmets</td>
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<td>119,643</td>
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<td>120,435</td>
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Except where noted below, all charges have been increased by 3% each year.

Monkeys: Thirty-five newborn rhesus macaques will be used over the five years of the grant period.

Goggles: Monkeys will be fitted with lightweight fiberglass helmets that serve to hold the goggles in place over the monkeys’ eyes. The estimated cost per monkeys is $100; this price includes the assistance of the machine shop at Yerkes in the manufacturing of the helmets and goggles, the Fresnel prism lenses, the plano spectacle lenses, and all of the other materials that are required for the goggle and helmet setup.

Yearly Costs: Year 1 = $900; Year 2 = $900; Year 3 = $900; Year 4 = $800; Year 5 = $0 (note: no yearly increase).

Ophthalmic Assessments: Prior to a monkey’s assignment to this study, it will undergo a complete ophthalmic examination by a pediatric ophthalmologist to ensure that the eyes are normal and healthy. The costs of the pre-screening, which includes the support of the veterinary staff for anesthesia is $70 per ½ hour or $140/hour. It is estimated that a ½ hour will be required to perform a single assessment.

Yearly Costs: Year 1 = $630; Year 2 = $648; Year 3 = $675; Year 4 = $624; Year 5 = $0.

Consortium

Fixed Fee (SBIR/STTR Only)
9 pages redacted--biosketches omitted as requested
RESOURCES AND ENVIRONMENT

FACILITIES: Mark the facilities to be used at each performance site listed in Item 9, Face Page, and briefly indicate their capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Use “Other” to describe the facilities at any other performance sites listed in Item 9 on the Face Page and at sites for field studies. Use continuation pages if necessary. Include an explanation of any consortium/contractual arrangements with other organizations.

Laboratory:

Clinical: n/a

Animal:

Computer:

A Macintosh and Dell PC running Cambridge Electronic Designs software is used for stimulus and reward delivery and eye movement recording. The software operates in conjunction with a CED 1401 computer interface and a digital eye-on-target detection circuit of our design. A PowerMacintosh G3 running CED and Igor software is used for off-line analysis. A Macintosh IIci using NUDIVA software supplied by A.M.Norcia of the Smith-Kettlewell Institute controls VEP stimulus delivery and recording. A PowerMacintosh G4 is also available for analysis and word processing. A Laser Writer is networked to the computers to print tracings of trials and data sets. A Toshiba laptop computer on a portable cart is used for training the animals to perform the LED dimming detection task. The items are located in our eye movement/VEP laboratory space. Within the neuroanatomy laboratory, a Macintosh G4 running Soft Solutions, NIH Image and Adobe Photoshop software, and a Pentium III coupled to digital imaging microscopes are available for tissue analysis and preparation of anatomic figures.

Office:

An office, approximately 150 sq. ft., is available for use by the PI.

Other:

The PI has a secretary for typing of manuscripts, etc. in an office suite in Children’s Hospital. The laboratory uses a fully equipped departmental printing darkroom. We have access to machine and electronic shops in the medical school for fabrication, modification and repair of equipment on a fee-for-service basis.

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

Available equipment includes a binocular magnetic scleral search coil system (CNC Engineering, Seattle WA) with an optical bench, scanners, and lasers for delivering stationary or moving targets and recording from alert animals; operating microscope; three research microscopes (connected to computers and equipped with video and still cameras); stereo microscope; sliding microtome for cutting frozen sections; Vibratome; assorted stereotaxic equipment and microdrives; Picospritzer.
RESOURCES

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate the performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Under "Other," identify support services such as machine shop, electronics shop, and specify the extent to which they will be available to the project. Use continuation pages if necessary.

Clinical: Five full-time vets supervise the clinical care. Rotating assignments assure that one of the vets is available at all times, 7/24. Each vet has several years of experience in dealing with infant monkeys reared with a variety of manipulations of visual input, including monkeys reared with goggles or reared with special housing to restrict visual input. A registered nurse and 7 vet-techs, experienced in working with primates, are also available and assist with ophthalmic exams.

Animal: Healthy newborn rhesus monkeys will be available for this project from the Yerkes Research Center Breeding

Computer: Three state-of-the-art IBM PC-computers and a Sun Spare-station (UNIX) computer are available for data collection, storage, and analysis for this study. Four computer service professionals are located at Yerkes. Additional computers and computing services, including statistical packages and interfaces to worldwide computer networks are available through the Emory University Computing Center.

Other:

The manufactures, on-site the goggles and helmets with the parameters that are appropriate for monkeys.

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

To perform ophthalmic assessments, the Division's Exam Rooms are equipped with Kowa portable slit lamp, a Kowa fundus camera, a Topcon slit lamp, a Terry quantitative surgical keratometer; an Alcon handheld automcrometer; a Sonomed A-scan ultrasound unit custom programmed for measuring the small eyes of monkeys; rechargeable retinoscopes; a complete trial lens set; a refractive bar; and both direct and indirect ophthalmoscopes.
A. **Specific Aims**

The first goal of this research proposal is to determine the age at which treatment of strabismus in macaques is capable of repairing behavioral abnormalities found in both humans and monkeys with infantile esotropia (Hypotheses 1-3). If the animals show behavioral recovery they will be considered to be an appropriate model for advocating prompt strabismus repair in human infants. The second goal of this research is to determine if the functional repair is accompanied by structural repair of visual cortex (Hypothesis 4). Precise behavioral-neuroanatomic correlation of this kind is not feasible in human infants. The experiments proposed in Hypotheses 1-4 are the logical outgrowth of the P.I.'s progress report which describes the functional-structural abnormalities caused by delayed repair and prevented by early repair of infantile strabismus. The proposed research uses methods similar to those used to generate the findings of the Progress Report, but applies the methods to help answer new questions bearing on a controversy of clinical importance: the efficacy and timing of early strabismus repair. The following specific hypotheses will be tested in each animal:

**Hypothesis 1.** Macaques with strabismus-onset at birth have maldeveloped pursuit eye movements that can be repaired if the strabismus is corrected by age 6-9 weeks.

**Hypothesis 2:** Macaques with strabismus onset 3 wks after birth have maldeveloped pursuit; the maldevelopment can be repaired if the duration of strabismus does not exceed 3 weeks.

**Hypothesis 3:** Strabismic macaques have maldeveloped short-latency ocular following and vergence eye movements that can be repaired if the strabismus is corrected within the time-frames specified in Hypotheses 1 and 2.
Hypothesis 4: Strabismic macaques have structural abnormalities in visual cortex that: a) correlate with their pursuit, ocular following and vergence behavioral "indicators", and b) can be repaired by early realignment of the eyes: the earlier the realignment, the greater the neuroanatomic recovery. Using

B. Background and Significance

Esotropia (convergent misalignment of the visual axes) represents over 90% of all strabismus that appears in human infancy. Infantile esotropia can be considered to be the paradigmatic form of developmental strabismus in primates, as it also occurs naturally in macaque monkeys. The PI treats hundreds of children each year who have infantile esotropia and has tested naturally and artificially-strabismic macaque monkeys to document that they exhibit the behavioral deficits of esotropic children (Figure 1). Hypotheses 1-4 of this proposal will determine whether very early repair of esotropia is capable of producing functional and neuroanatomic cures. The work will help answer questions posed by parents and pediatricians, by ophthalmologists and optometrists, and by basic scientists concerned with brain maldevelopment and optimal treatment of cross-eyed infants.

2.1 Infantile Strabismus

The behavioral deficits of early-onset esotropia, whether naturally occurring or experimentally induced, appear to be remarkably similar in human and monkeys infants. The artificial strabismus in monkeys can be created by surgical weakening, chemodenervation of the lateral rectus muscles, by daily alternating occlusion, or by prism goggles. As is true of human infants who are esotropic, the majority of these esotropic monkey infants alternate viewing from eye to eye and have good visual acuity (no significant amblyopia) in each eye. Both natural and artificially esotropic monkeys display a constellation of functional deficits (or "indicators"), including asymmetric pursuit eye movements, abnormal motion sensitivity and subnormal disparity sensitivity. The findings of our Progress Report indicate that these deficits are linked to abnormal patterns of connectivity in visual area V1. Studies of human infants whose strabismus was surgically corrected in the first months of life suggest that these functional deficits may be repaired by early realignment.
Pursuit Eye Movements: Smooth pursuit eye movements in infantile esotropes are directionally asymmetric: under conditions of monocular viewing, horizontal pursuit gain (eye velocity/target velocity) is normal for targets moving nasalward in the visual field, and subnormal for targets moving temporalward. The asymmetry is exhibited transiently in normal human and monkey infants before the onset of binocularity, but persists permanently in primates who develop infantile-onset strabismus. The directional asymmetry is not seen in primates who had onset of strabismus after infancy. Beyond its utility as an indicator, the asymmetry merits study as a phenomenon of abnormal binocular development. The directional asymmetry occurs independent of where the eye is in the orbit or where the target is in the visual field, but the asymmetry inverts instantaneously 180° with a change of fixing eye (Figure 2). The observations suggest a linkage between abnormal binocular connections and abnormal motion processing in V1 that affects downstream pursuit pathways in an early, but as yet undefined, critical period for motor development. Clinical reports of a small number of human infants whose strabismus was surgically corrected by 4 mos (the equivalent of age 4 wks in monkey) suggest that the tracking asymmetry persists, but may be less severe than that reported in infants whose surgical repair is delayed to age 1 yr or beyond.

Ocular Following Movements: Short latency ocular following responses (OFR) help to stabilize vision immediately after and during eye/head movement. OFR aids clear vision by reducing slip of retinal images immediately after saccades (compensating for post-saccadic drift, or glissades) and during vestibulo-ocular reflex movements (compensating for small errors in a less-than-perfect VOR response). OFR appears to be optimally suited to tracking of foreground targets in the natural world. Contrary motion in the peripheral visual field (motion parallax of the background) boosts the tracking response evoked by foreground motion in the central field (anti-phase enhancement). OFR is also modulated normally by binocular disparity, which operates to aid tracking of an object in the plane of fixation (the horopter) and to ignore the conflicting motion of objects closer or farther away.

Infantile strabismus could impair operation of OFR for at least two, immediately-obvious reasons: (1) poor disparity sensitivity and/or abnormal vergence would interfere with selection of which target to track, the near or the far, and (2) directional asymmetries or adaptations of motion sensitivity would boost the response to retinal slip in one direction but weaken the response in the other. OFR has been shown to be strong but abnormally modulated in a stereo-blind, adult human with non-infantile strabismus.

Disparity Vergence Eye Movements: Vergence eye movements are used to align the foveae of each eye on the object of regard, and comitant, infantile strabismus represents a maldevelopment of the vergence system, though the specific site of the defect is not known. In humans, subnormal disparity vergence correlates strongly with subnormal stereopsis, implying that both are driven by binocular neurons in visual cortex. Stimulation of occipital cortex evokes vergence in monkeys and sectioning of the corpus callosum abolishes disparity vergence when the target is positioned to project the retinal image to opposite striate cortices. Disparity-selective neurons implicated in the control of vergence have been found in many cortical visual areas in monkey and fall generally into “near” and “far” subtypes, sensitive to crossed and uncrossed disparities and to individual disparities. These neurons respond best to disparities of at most a few degrees, and normal monkeys have the strongest short-latency vergence responses for disparities <3°. Masson et al. have shown that patterns containing binocular disparity act as a powerful stimulus to short-latency vergence in both humans and monkeys, even if the patterns cannot evoke a percept of binocular fusion because they are composed of anticorrelated dots. Taken together, these findings indicate that short-latency vergence is driven by disparity-sensitive neurons at the earliest (i.e. pre-stereoscopic) stage of binocular processing -- visual area V1.
In the results of the Progress Report we used short-latency vergence as a probe of V1 disparity sensitivity in strabismic and normal juvenile and adult macaques. Our findings suggest that early repair of strabismus restores normal short-latency vergence, and delayed-repair permanently impairs vergence.

**V1 Neuroanatomy:** Monkeys who have delayed repair or unrepaired, natural or artificial strabismus have striking structural and metabolic abnormalities in V1. The major structural deficit is a paucity of horizontal connections between ODCs of opposite-ocularity, and a strong preference for connections to ODCs of the same ocularity.\(^{119, 120, 122}\) The defect of binocular connectivity is apparent in layer 4B and in inter-patch compartments of layers 2/3. The defects relate systematically to ocular motor and motion VEP abnormalities in that the most severe defects of horizontal connections are observed in the animals with the most severe behavioral impairments. The metabolic defect is suppression of cytochrome oxidase (CO) activity in ODCs of opposite-ocularity, which may extend across foveal V1 of both hemispheres or appear as a suppression scotoma in one retinotopic region.\(^{55, 121}\) Abnormal binocularity also produces more subtle changes in the cytoarchitecture of ODCs in strabismic macaques: alterations in CO staining, as well as calbindin and neurofilament protein labeling, are most apparent at the borders of ODCs and may prove to be markers for loss of binocular neurons in these zones.\(^{39, 55}\)

**2.2 Gaps In Our Knowledge Which the Proposed Research Will Fill**
2.3 Relation of the Specific Aims to Long-Term Research Objectives

The Specific Aims stress correlations between ocular motor behavior and V1 anatomy because the findings of our progress report suggest plausible V1 mechanisms for these strabismic behaviors. An investigation of maldevelopment and repair in strabismus should logically begin in V1, since (a) V1 is the first locus in the CNS containing binocular visual neurons and (b) V1 is an early component in the pathways for pursuit, disparity vergence and the OFR. An advantage of studying V1 is that considerable knowledge exists from other laboratories regarding the innate development and postnatal refinement of ODCs and their horizontal connections. The long-term objective of this research is to reveal the neural mechanisms that explain each of the behavioral deficits of infantile strabismus and the time in development at which they occur. The ultimate aim is to develop refined neuropharmacologic and surgical treatments to achieve functional cures.

2.4 Health Relevance of the Specific Aims

Strabismus is one of the most prevalent health problems among children in the Western hemisphere, affecting five in every 100 U.S. citizens, or some 12 million persons in a population of 245 million. Of all subtypes of human strabismus, infantile strabismus may be the most important but least understood. It is important to clinicians because it is difficult to treat, often requiring multiple surgical procedures in order to restore proper eye alignment. It is important to vision scientists because it is accompanied by profound maldevelopments of stereopsis, motion processing, eye tracking, and ocular fixation. The study proposed here could lead to a fundamental advance in understanding the biological mechanisms of this disorder.

C. Progress Report

Publications Related to This Grant (12/98-present, published or submitted).


Wong, A.M.F., Lueder, G.T., Burkhalter, A. and Tychsen, L.: Anomalous retinal correspondence:


Aim 1: Macaques who have infantile strabismus have maldeveloped pursuit eye movements that can be repaired by early realignment of the eyes; the earlier the realignment, the greater the ocular motor recovery.
Delayed repair of optical strabismus (prism-rearing for 12 wks or longer) causes permanent pursuit/OKN asymmetry: the longer the delay, the more severe the asymmetry.
Delayed repair of optical strabismus causes permanent eye misalignment (heterotropia), latent fixation nystagmus and dissociated vertical deviation: the longer the delay, the more severe the abnormalities.
Aim 2: Strabismic macaques have abnormalities of random-dot stereogram VEP function and motion VEP function that can be repaired by early realignment of the eyes; the earlier the realignment, the greater the electrophysiological recovery.
Delayed repair causes subnormal binocular disparity sensitivity.

Optical strabismus causes fixation preferences but does not produce significant amblyopia, as measured by spatial sweep VEP.

Aim 3: Strabismic macaques have structural abnormalities in visual cortex that: a) correlate with their pursuit eye movement and VEP behavioral "indicators", and b) can be repaired by early realignment of the eyes; the earlier the realignment, the greater the neuroanatomic recovery.
Delayed-repair monkeys have a reduced number of binocular excitatory connections, but an increased number of inhibitory (GABA) connections in V1.
D. **Research Design and Methods**

**Hypothesis 1:** Macaques with strabismus-onset at birth have maldeveloped pursuit eye movements that can be repaired if the strabismus is corrected by age 6-9 wks.

**C. Hypothesis** - Macaques who have optically-induced strabismus from birth have directional asymmetries of horizontal pursuit that can be repaired if the strabismus is corrected by age 6-9 wks.

1.1.2. **Rationale** - Humans and monkeys with infantile strabismus have a directional asymmetry of horizontal pursuit initiation favoring nasalward target motion. Early repair of strabismus in human infants is believed to reduce the asymmetry, implying that the critical period for pursuit visuomotor circuits in human begins and ends early in development. Early repair (by age 3 wks) of optical strabismus in monkey restores normal pursuit symmetry, whereas delay until age 12 wks causes permanent asymmetry. This experiment will determine whether repair can be delayed until age 6-9 wks without causing pursuit asymmetry.
Hypothesis 2: Macaques with strabismus-onset 3 wks after birth have maldeveloped pursuit; the maldevelopment can be repaired if the duration of strabismus does not exceed 3 wks.

2.1 Hypothesis - Macaques with strabismus-onset 3 wks after birth (~3 mos human) will have horizontal pursuit asymmetry if repair is delayed more than 3 wks from onset.

2.1.3. Method of Data Collection – Identical to the Methods of 1.1.3 above.

2.1.4. Data Analysis – Identical to the Methods of 1.1.4 above.

2.1.5. Expected Result -

Hypothesis 3: Strabismic macaques have maldeveloped short-latency ocular following and vergence eye movements that can be repaired if the strabismus is corrected within the time-frames specified in Hypotheses 1 and 2.

3.1 Hypothesis – Macaques who have onset of strabismus at or before age 3 wks have asymmetries of horizontal ocular following when viewing monocularly. The asymmetries can be prevented or repaired: (a) if stra-
bismus with onset at birth is corrected by age 6-9 wks, or (b) if strabismus with onset at 3 wks is corrected within 3 wks (i.e. by age 6 wks).

3.1.1 Rationale -

3.1.2 Methods of Data Collection -
3.1.3 Data Analysis –

3.1.4 Expected Result –

3.2 Hypothesis - Macaques who have onset of strabismus at or before age 3 wks have abnormal short-latency disparity vergence eye movements. The abnormalities can be prevented or repaired: (a) if strabismus with onset at birth is corrected by age 6-9 wks, or (b) if strabismus with onset at 3 wks is corrected within 3 wks (i.e. by age 6 wks).

3.2.1 Rationale - The results of our Progress Report show that monkeys who have delayed repair of optical strabismus have subnormal and even “wrong way” disparity vergence responses. Early correction of the strabismus restores normal responsiveness. We will attempt to define more precisely whether the period in which disparity vergence maldevelopment occurs coincides with the period of stereoscopic development (age 3-9 wks in infant macaques). The disparity sensitivity of each animal revealed in these experiments will aid interpretation of the experiment of 3.1 in which OFR is modulated by binocular disparity.

3.2.2 Methods of Data Collection –

3.2.3 Data Analysis –
3.3 Potential Pitfalls and Contingency Experiments - 

3.4 Procedures Common to the Experiments under Hypothesis 1 - 3

3.4.1. Prism Goggle Fitting and Rearing - 

3.4.2. Initial Visual Training -
3.4.3 Coil and Head Restraint Surgery

3.4.4 Eye Movement Recording
Hypothesis 4: Strabismic macaques have structural abnormalities in visual cortex that: a) correlate with their pursuit, ocular following and vergence behavioral "indicators", and b) can be repaired by early realignment of the eyes; the earlier the realignment, the greater the neuroanatomic recovery.

4.1. Hypothesis - The earlier the age-at-repair of eye misalignment in infancy, the greater the number of horizontal axonal projections that interconnect ODCs of opposite-ocularity.

4.1.1. Rationale – A matter of clinical controversy is the age at which strabismus surgery should be performed in infants with esotropia in an attempt to restore normal levels of binocular vision (fusion and stereopsis).7, 60, 143 The results of our Progress Report show normal binocular V1 connectivity in monkeys whose strabismus was repaired by age 3 wk (~3 mon human equivalent) and poor connectivity when repair was delayed until age 12 wk (~1 yr human). In this set of experiments we will define more precisely the upper age limit for neuroanatomic repair of V1 in monkeys.

4.1.2. Methods of Data Collection -

4.1.3. Data Analysis –
4.2. Hypothesis – The shorter the duration of eye misalignment in infancy, the greater the number of horizontal axonal projections that interconnect ODCs of opposite-ocularity.

4.2.1. Rationale – A substantial number of human infants do not have onset of constant strabismus before age 3 mos. Clinical studies of a small number of these children suggests that good-to-excellent restoration of binocular vision may be achieved when strabismus repair is carried out after age 4 mos, so long as the duration of strabismus did not exceed 2-3 mos. To test this clinical finding, we will determine how duration of strabismus before repair influences neuroanatomic outcome when onset of strabismus does not occur until age 3 wks (the equivalent of 3 mos in children).

4.2.2. Methods of Data Collection – Identical to Methods of 4.1.2 above.

4.2.3. Data Analysis – Identical to Data Analysis of 4.1.3 above.
4.3 Hypothesis - Delayed repair monkeys have an increased number of inhibitory (GABAergic) terminals on pyramidal cells that form connections between ODCs of opposite-ocularity (Figure 14).

4.3.1 Rationale -
4.3.3. Data Analysis -

4.3.4. Expected Result -

4.4. Potential Pitfalls and Contingency Experiments -
4.5 Methods Common to the Experiments of Hypothesis 4
4.6 Time Table for Experiments
E. **Human Subjects**

No human subjects will be used in this project.

F. **Vertebrate Animals**

**Justification for selection of a higher species** - Our choice of macaque is based on several advantages they have over other experimental animals. Foremost is the fact that macaques have a time course for postnatal development of binocular vision closely mimicking that of human, albeit on a compressed time scale. Macaques are widely used in behavioral studies of visual function, and for that reason alone it is important to understand the organization of their visual cortex. The specific ocular motor issues we propose to address can only be answered by studying alert, behaving animals. We will be using *M. nemestrina*, and *M. mulatta*. As far as is known, there are no major differences in the organization of visual cortex among different macaque species.

The experimental questions we are asking can only be answered by study of a species with a well developed fovea centralis and fine smooth pursuit eye movements. These criteria exclude rodents, rabbits, or cats as suitable animals for the proposed research. The macaque ocular motor system has been shown to be highly similar to that of humans. In particular, visually-guided eye movements and the binocular visual cortex, which are of primary interest in the present proposal, have been shown to be organized in highly-similar fashion in human and macaque. Infantile strabismus in the macaque closely mimics the esotropia that develops in human infants, and as such these animals represent an appropriate animal model for elucidation of the neural mechanisms that could ultimately explain human strabismus. The use of a non-human model of strabismus allows for tight correlation of precise behavioral and neuroanatomical results not possible in studies of children. Finally, the great corpus of Judeo-Christian moral teaching, which has guided Western civilization for over two-thousand years, firmly endorses the proper and humane use of animals, including monkeys, to further the understanding of human disease and alleviate human suffering.
G. Literature Cited


February 25, 2002

Dorothy C. Yates, Director
Sponsored Projects Services
Washington University
Campus Box 1054
One Brookings Drive
St. Louis, MO 63130

RE: Subcontract Proposal “Early Functional and Structural Repair in Macaques”
Principal Investigator (Emory): __________
Principal Investigator (WU): Lawrence Tychsen

Dear Ms. Yates:

The purpose of this letter is to inform you that the appropriate programmatic and administrative personnel at Emory University involved in this grant application are aware of the PHS consortium policy and are prepared to establish the necessary inter-institutional agreement consistent with that policy.

Please contact my office at the number listed below should you have any questions or concerns.

Sincerely,

Jackie Bendall
Assistant Director for Research
TYPE OF APPLICATION (Check all that apply.)

☐ NEW application. (This application is being submitted to the PHS for the first time.)

☐ SBIR Phase I ☐ SBIR Phase II: SBIR Phase I Grant No. ☐ SBIR Fast Track
☐ STTR Phase I ☐ STTR Phase II: STTR Phase I Grant No. ☐ STTR Fast Track

☐ REVISION of application number:

(This application replaces a prior unfunded version of a new, competing continuation, or supplemental application.)

☐ COMPETING CONTINUATION of grant number: 5 R01 EY10214-07

☐ SUPPLEMENT to grant number:

(This application is for additional funds to supplement a currently funded grant.)

☐ CHANGE of principal investigator/program director.

Name of former principal investigator/program director:

☐ FOREIGN application or significant foreign component.

1. PROGRAM INCOME (See instructions.)

All applications must indicate whether program income is anticipated during the period(s) for which grant support is requested. If program income is anticipated, use the format below to reflect the amount and source(s).

<table>
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<th>Budget Period</th>
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2. ASSURANCES/CERTIFICATIONS (See instructions.)

The following assurances/certifications are made and verified by the signature of the Official Signing for Applicant Organization on the Face Page of the application. Descriptions of individual assurances/ certifications are provided in Section III. If unable to certify compliance, provide an explanation and place it after this page.

☒ Human Subjects; ☒ Research Using Human Pluripotent Stem Cells
☒ Research on Transplantation of Human Fetal Tissue ☒ Women and Minority Inclusion Policy ☒ Inclusion of Children Policy ☒ Vertebrate Animals

3. FACILITIES AND ADMINISTRATIVE COSTS (F&A)/INDIRECT COSTS. See specific instructions.

☒ DHHS Agreement dated: 12/04/01; provisional as of 7/1/04.

☐ No Facilities And Administration Costs Requested.

☐ DHHS Agreement being negotiated with Regional Office.

☐ No DHHS Agreement, but rate established with Date

CALCULATION* (The entire grant application, including the Checklist, will be reproduced and provided to peer reviewers as confidential information. Supplying the following information on F&A costs is optional for for-profit organizations.)

<table>
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<tr>
<th>Period</th>
<th>Amount of base $</th>
<th>Rate applied</th>
<th>% F&amp;A costs</th>
<th>Amount $</th>
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a. Initial budget period: 261,758 x Rate applied 53 % = F&A costs $ 138,732
b. 02 year Amount of base $ 242,328 x Rate applied 53 % = F&A costs $ 128,434
c. 03 year Amount of base $ 268,743 x Rate applied 53 % = F&A costs $ 142,434
d. 04 year Amount of base $ 278,490 x Rate applied 53 % = F&A costs $ 147,600
e. 05 year Amount of base $ 279,658 x Rate applied 53 % = F&A costs $ 148,219

TOTAL F&A Costs $ 705,419

*Check appropriate box(es):

☐ Salary and wages base ☒ Modified total direct cost base ☐ Other base (Explain)

Explanation (Attach separate sheet, if necessary):  

4. SMOKE-FREE WORKPLACE ☒ Yes ☐ No (The response to this question has no impact on the review or funding of this application.)