Notice of Award

RESEARCH
Department of Health and Human Services
National Institutes of Health

NATIONAL EYE INSTITUTE

Federal Award Date: 02/26/2016

Grant Number: 1R01EY026568-01
FAIN: R01EY026568

Principal Investigator(s):
VALLABH E DAS, PHD

Project Title: Binocular Coordination of Eye Movements

Ms. Griffing, Angelyn C.
Senior Research Administrator
4800 Calhoun
316 E. Cullen Building
Houston, TX 772042015

Award e-mailed to: uhawards-l@listserv.uh.edu

Period Of Performance:
Budget Period: 04/01/2016 – 03/31/2017
Project Period: 04/01/2016 – 03/31/2019

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $376,250 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF HOUSTON in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Eye Institute of the National Institutes of Health under Award Number R01EY026568. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

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WILLIAM W DARBY
Grants Management Officer
NATIONAL EYE INSTITUTE

Additional information follows

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SECTION I – AWARD DATA – 1R01EY026568-01

Award Calculation (U.S. Dollars)

Federal Direct Costs	$250,000
Federal F&A Costs	$126,250
Approved Budget	$376,250
Total Amount of Federal Funds Obligated (Federal Share)	$376,250
TOTAL FEDERAL AWARD AMOUNT	$376,250
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$376,250

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
1	$376,250	$376,250
2	$376,250	$376,250
3	$376,250	$376,250

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name:	Vision Research
CFDA Number:	93.867
EIN:	1746001399A3
Document Number:	REY026568A
PMS Account Type:	P (Subaccount)
Fiscal Year:	2016

IC	CAN	2016	2017	2018
EY	8472436	$376,250	$376,250	$376,250

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: 5C /HXA / **OC**: 414A / **Released**: [redacted] 02/22/2016
Award Processed: 02/26/2016 12:08:19 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 1R01EY026568-01

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 1R01EY026568-01

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain

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references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2. As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase VI Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots.

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01EY026568. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:
Additional Costs

SECTION IV – EY Special Terms and Conditions – 1R01EY026568-01

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MODULAR GRANTS
This is a Modular Grant Award without direct cost categorical breakdowns issued in accordance with the guidelines published in the NIH Grants Policy Statement at: http://grants.nih.gov/grants/policy/nihgps/nihgps.pdf. Recipients are required to allocate and account for costs related to this award by category within their institutional accounting system in accordance with applicable cost principles.

GRADUATE STUDENT COSTS
In accordance with the Notice: NOT-OD-02-017 entitled, "GRADUATE STUDENT COMPENSATION" published on December 10, 2001, in the NIH Guide for Grants and Contracts, total direct costs (salary, fringe benefits and tuition remission) for graduate students are provided at a level not to exceed the NIH maximum allowable amount (zero level of the Ruth L. Kirschstein National Research Service Award stipend in effect at the time of the competing award). Support recommended for future years has been adjusted accordingly, if applicable. The full guide Notice describing the level of compensation allowed for a graduate student can be found at: http://grants.nih.gov/grants/guide/notice-files/NOT-OD-02-017.html.

SALARY CAP
None of the funds in this award shall be used to pay the salary of an individual at a rate in excess of the applicable salary cap. Therefore this award and/or future years are adjusted accordingly, if applicable. Current salary cap levels can be found at the following URL: http://grants1.nih.gov/grants/policy/salcap_summary.htm.

CHANGE IN EFFORT FOR SENIOR/KEY PERSONNEL REMINDER:
Grantees are responsible for reporting any significant changes in effort of key personnel if the status is defined as reduction of time devoted to the project by 25 percent, or more from the level that was approved at the time of initial competing year award (example: a proposed change from 2 calendar months effort to 1.5 calendar or fewer months' effort would require prior approval), absence from the project for any continuous period of three months, or withdrawal from the project. Prior approval is only required for a change in status for the PD/PI or other senior/key personnel specifically named in the NoA.

PRIOR APPROVAL
Requests which require the prior approval of the NEI must be submitted in writing to the Grants Management Specialist. All requests should reference the complete grant number, 1 R01 EY 026568 - 01, and must be signed by the authorized official of the business office of the grantee organization and by the principal investigator.

ROLES AND RESPONSIBLITIES
If you need assistance from the National Eye Institute (NEI) during the course of this grant, please contact the grants management and program staff listed on the Notice of Grant Award (NGA). The telephone numbers of these individuals, as well as other extramural staff members of the NEI, can be located on the NEI web site, http://www.nei.nih.gov. The grants management and program staff members work closely with one another through all phases of the project to facilitate the award and the administration of the grant. Their functions are defined as follows:

GRANTS MANAGEMENT CONTACT: The Grants Management Specialist is responsible for all business management matters associated with the review, negotiation, award, and administration of grants. Grants Management Specialists serve as the focal point for receiving and responding to all questions and correspondence related to business management and policy matters, such as correspondence giving or denying any prior approval required by Public Health Service (PHS) policy or special Terms and Conditions of Award, transfer of the grant to another institution, a change in the period of support, or any action which commits, or may result in committing the NEI to a change in the amount of funding.

PROGRAM CONTACT: The Program Director is responsible for all scientific and technical matters associated with the grant. The program official reviews and monitors scientific progress of the project and provides advice and assistance relative to all technical problems to ensure that the scientific objectives of the research program can be pursued effectively and successfully. All questions or correspondence dealing with research progress, changes in research direction, unique scientific opportunities, or any other scientific needs should be addressed to the Program Director.

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STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Pamela Bobbitt
Email: bobbittp@mail.nih.gov **Phone**: 301-451-2020 **Fax**: 301-496-9997

Program Official: Houmam H Araj
Email: ha50c@nih.gov **Phone**: (301) 451-2020 **Fax**: (301) 402-0528

SPREADSHEET SUMMARY

GRANT NUMBER: 1R01EY026568-01

INSTITUTION: UNIVERSITY OF HOUSTON

Budget	Year 1	Year 2	Year 3
TOTAL FEDERAL DC	$250,000	$250,000	$250,000
TOTAL FEDERAL F&A	$126,250	$126,250	$126,250
TOTAL COST	$376,250	$376,250	$376,250

Facilities and Administrative Costs	Year 1	Year 2	Year 3
F&A Cost Rate 1	50.5%	50.5%	50.5%
F&A Cost Base 1	$250,000	$250,000	$250,000
F&A Costs 1	$126,250	$126,250	$126,250

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PI: **DAS, VALLABH E**	Title: Binocular Coordination of Eye Movements	
Received: 06/03/2015	FOA: PA13-302	Council: 01/2016
Competition ID: FORMS-C	FOA Title: RESEARCH PROJECT GRANT (PARENT R01)	
1 R01 EY026568-01	Dual:	Accession Number: 3826467
IPF: 1449402	Organization: UNIVERSITY OF HOUSTON	
Former Number:	Department: College of Optometry	
IRG/SRG: SPC	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 250,000 Year 2: 250,000 Year 3: 250,000 Year 4: 250,000 Year 5: 250,000	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
Vallabh Das	University of Houston	PD/PI

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OMB Number: 4040-0001
Expiration Date: 06/30/2016

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier
746001399

b. Agency Routing Number

2. DATE SUBMITTED
2015-06-03

Application Identifier

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION

Organizational DUNS*: 036837920

Legal Name*: University of Houston
Department:
Division:
Street1*: Office of Contracts and Grants
Street2: 316 E. Cullen Building
City*: Houston
County: Harris
State*: TX: Texas
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 77204-2015

Person to be contacted on matters involving this application
Prefix: Ms. First Name*: Angelyn Middle Name: C. Last Name*: Griffing Suffix:
Position/Title: Senior Research Administrator
Street1*: 4800 Calhoun
Street2: 316 E. Cullen Building
City*: Houston
County: Harriis
State*: TX: Texas
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 772042015
Phone Number*: 7137439284 Fax Number: Email: eagriff2@central.uh.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 746001399

7. TYPE OF APPLICANT* H: Public/State Controlled Institution of Higher Education

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

● New ❍ Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Binocular Coordination of Eye Movements

12. PROPOSED PROJECT
Start Date* 04/01/2016
Ending Date* 03/31/2021

13. CONGRESSIONAL DISTRICTS OF APPLICANT
TX-018

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SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. First Name*: Vallabh Middle Name: Easwara Last Name*: Das Suffix:

Position/Title: Professor
Organization Name*: University of Houston
Department: College of Optometry
Division:
Street1*:
Street2:
City*: Houston
County: Harris
State*: TX: Texas
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 77204-2015
Phone Number*: 713-743-3292 Fax Number: Email*: vdas@central.uh.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$1,881,250.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$1,881,250.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR

○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: Ms. First Name*: Angelyn Middle Name: Last Name*: Griffing Suffix:

Position/Title*: Senior Research Administrator
Organization Name*: University of Houston
Department: Offfice of Contacts & Grants
Division: Research
Street1*: 316 E. Cullen Building
Street2: 4800 Calhoun
City*: Houston
County: Harris
State*: TX: Texas
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 77204-2015
Phone Number*: 713-743-9284 Fax Number: 713-743-9227 Email*: eagriff2@central.uh.edu

Signature of Authorized Representative*
Angelyn Griffing

Date Signed*
06/03/2015

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:Das_StrabismusSC_Cover_Letter1011854061.pdf

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424 R&R and PHS-398 Specific Table Of Contents

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OMB Number: 4040-0010
Expiration Date: 06/30/2018

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	University of Houston
Duns Number:	036837920
Street1*:	
Street2:	
City*:	Houston
County:	Harris
State*:	TX: Texas
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	77204-2015
Project/Performance Site Congressional District*:	TX-018

File Name

Additional Location(s)

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OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ○ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ○ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6

If NO, is the IRB review Pending? ○ **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ○ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ○ Yes ● No

IACUC Approval Date: 05-23-2014

Animal Welfare Assurance Number A-3136-01

3. Is proprietary/privileged information included in the application?* ○ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ○ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Das_StrabismusSC_Abstract1011854062.pdf
8. Project Narrative*	Das_StrabismusSC_Narrative1011854063.pdf
9. Bibliography & References Cited	Das_StrabismusSC_GrantFinal_Bibliography1011854243.pdf
10.Facilities & Other Resources	Das_StrabismusSC_Resources1011854064.pdf
11.Equipment	Das_StrabismusSC_Major_Equipment1011854065.pdf

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Abstract

The overall goal of research in our laboratory is to investigate the post-natal development of the ocular motor system in normal monkeys and monkeys reared under conditions to induce developmental problems such as ocular misalignment (strabismus). Strabismus of sensory origin is a significant public health problem since it affects 2-5% of the infant population. Previously we have shown, in an animal model for strabismus, that a slow vergence eye movement pathway involving projections from the midline cerebellar nuclei to the supraoculomotor area and thereafter the medial rectus motoneurons in the oculomotor nucleus partially drives the steady state misalignment. In the present investigation of strabismus mechanisms, we propose to investigate the neural basis for three strabismus properties – 1) eye misalignment, 2) fixation instability and 3) fixation-switch. Each of these strabismus properties implicates an important oculomotor control structure, the Superior Colliculus (SC), and our plan is to couple behavioral studies with neurophysiological investigation of SC contributions. The experiments are organized into three specific aims. In specific aim 1, we propose single unit recording studies and muscimol inactivation studies in the rostral SC with the goal of determining contribution of the rostral SC in defining state of eye misalignment. The motivation for these studies is that human clinical and animal neural recording and lesion studies suggest that the rostral SC is important in control of slow vergence movements including alignment of the eyes. In aim 2, we propose single-unit recording studies and muscimol inactivation studies of the rostral SC to determine its role in fixation instability, a problem frequently associated with strabismus and amblyopia. The motivation for these experiments is that studies in normal monkeys have proposed a framework where balanced activity across the two rostral colliculi promotes fixation. In aim 3, we propose single unit recording studies of neurons in the caudal SC to determine their role in fixation-switch (alternating saccade) behavior frequently observed in humans and monkeys with strabismus. We propose to test a framework wherein fixation-switch behavior is driven via the same neural mechanisms that drive target selection in normal monkeys. Studies will be performed in juvenile rhesus monkeys previously induced with a sensory form of strabismus by rearing them under special viewing conditions (optical prism rearing) for the first four months of their life. In summary, each of the specific aims in this project is likely to significantly advance our understanding of strabismus mechanisms and neural circuitry and has the potential to eventually help guide the development of rationally based therapies.

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PROJECT NARRATIVE

Ocular misalignment (strabismus) is a developmental disorder that affects a significant number of children born every year in the United States and around the world. A better understanding of neural mechanisms that are affected in the different forms of strabismus will help develop rationally based therapy. This particular project will combine various physiological methods and behavior investigation in an animal model to investigate neural correlates to oculomotor disruptions in strabismus including eye misalignment, fixation instability and fixation-switch behavior.

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Facilities and Resources Description

Laboratory:

We have approximately 400 square feet of dedicated laboratory space located on the [redacted] at the College of Optometry, University of Houston. The laboratory comprises 2 contiguous animal test booths each with a complete neurophysiology setup for testing in awake-behaving primates. Currently one animal setup is also configured for vestibular testing capabilities. Each animal setup contains a 400mm coil frame search-coil system (Primelec Industries) to precisely measure eye movements in the animals. The coil frame is designed to mount on the primate chair. Visual stimuli are projected using a rear-projection system (DepthQ projectors). One of the setups is also equipped with remote infrared photorefraction for measurement of accommodation. Equipment in each animal setup (test room) is hooked up to data acquisition and stimulus control setups mounted on standard 21inch racks outside of the recording booth. Space outside of the recording booths is available for conducting other activities such as preparation of electrodes and contact lens or goggle preparation for strabismus rearing experiments.

Clinical: N/A

Animal:
Infant animals are housed in special animal quarters at the University of Houston, College of Optometry in the [redacted] Infants live in temperature controlled isolates for the first 1-2 months before being moved to animal cages. Members of the Animal Care Operations group in consultation with members of our lab perform all activities associated with animal maintenance including formula feeding and monitoring of normal growth. The per diem rates included in our budget support these services. A fully equipped animal only surgical facility is available for all investigators. Housing for juvenile animals is immediately next to the lab allowing for easy and quick access to the animals for testing.

Computer:
We have dedicated computers for data acquisition and stimulus control. We use Windows PC based systems for stimulus control, data acquisition and analysis. The primary data acquisition computer for single-unit experiments in each setup is equipped with an AlphaLab SnR system that is responsible for acquiring single-unit and eye movement data, pre and post amplification of neuronal voltages and sorting of spike data. The AlphaLab system has inbuilt capabilities to deliver electrical stimulation via the same recording electrode. Stimulus control is carried out by a computer equipped with a BITS# stimulus generator platform from Cambridge Research Systems. Much of our data analysis is performed on dedicated PC's. These computers are heavily used for data analysis using specially written software. We are using Matlab (Mathworks) analytic environment and custom software for data anlysis. Currently, computer facilities related to data analysis are maintained by the P.I. with help as needed from the UHCO IT staff. All computers are connected to the campus broad-band network.

Office:
The PI has separate office space (100 square feet) [redacted] Students and staff associated with the lab also have separate office space on [redacted]

Other:
Machine and Electronics Shop: Machine shop support is available at the excellent machine shop at UHCO that is manned on a full-time basis by two experienced personnel. There is no cost to the investigator as the machine shop is supported by a Core grant to the College. However specialized projects are charged a fee for purchase of dedicated tools. Similarly, electronics support is available from an electronics shop that is manned by a full-time engineer. Services of the engineer are available for all investigators.

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MAJOR EQUIPMENT

We have all equipment necessary for eye movement and single unit recording studies of oculomotor system neural structures.

Setup 1 (Visual testing)

- 3-field search coil system from Primelec Industries (Switzerland) capable of measuring binocular eye movements. 400mm coil frame
- AlphaLab SnR system for acquisition of eye, target and unit signals, electrical stimulation, post-amplification and spike sorting.
- Electrode Positioning system along with dual electrode MT system from AlphaOmega Eng for simultaneous dual recording experiments.
- BITS# system (Cambridge Research Systems) along with Psychtoolbox 3.0 and MATLAB for generation of visual stimuli.
- Two custom remote infrared photorefractors (designed and incorporated with the help of Dr. Adrian Glasser, University of Houston). This equipment is necessary to measure eye accommodation in the animals.
- DepthQ projector capable of projecting frame sequential signals synced with LCD goggles.
- Tangent screen.
- Custom computerized reward setup.

Setup 2 (Visual and Vestibular testing)

- Identical setup as Setup 1 in all aspects except it includes 35 ft-lb continuous torque motor rate table from Neurokinetics for horizontal rotation vestibular testing. This setup does not have accommodation measurement facility.

Other equipment common to both setups

- Primate chairs
- Microscope
- Infant monkey isolates
- Refrigerator
- Weighing scale

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OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: Vallabh Middle Name Easwara Last Name*: Das Suffix:

Position/Title*: Professor
Organization Name*: University of Houston
Department: College of Optometry
Division:
Street1*: [redacted]
Street2:
City*: Houston
County: Harris
State*: TX: Texas
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 77204-2015

Phone Number*: 713-743-3292 Fax Number: E-Mail*: vdas@central.uh.edu

Credential, e.g., agency login: [redacted]

Project Role*: PD/PI Other Project Role Category:

Degree Type: PhD Degree Year: 1999

	File Name
Attach Biographical Sketch*:	Das_StrabismusSC_biosketch_Ver[illegible]1854244.pdf
Attach Current & Pending Support:	

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OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Vallabh Das

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Birla Institute of Technology and Science, Pilani, India	B.E. (Hons)	05/1992	Electronics & Instrumentation
Case Western Reserve University, Cleveland, OH	M.S	06/1995	Biomedical Engineering
Case Western Reserve University, Cleveland, OH	Ph.D.	06/1999	Biomedical Engineering
Yerkes National Primate Center, Emory University, Atlanta, GA	Post-doc	01/2002	Oculomotor Neurophysiology

A. Personal Statement

The focus of research in my laboratory is to investigate disruption of eye movement control in non-human primate models for strabismus. Strabismus is a common visual developmental disorder affecting 2-5% of all human infants. Though the exact etiology of strabismus is still unknown, it is clear that disruption of binocular visual information in infancy plays a critical role in development of strabismus. Many seminal behavioral, anatomical and physiological studies have revealed various aspects of visual sensory deficits that are associated with the strabismic condition. By the same token, we know relatively little about disruptions in neural oculomotor (eye movement) circuits, though these structures must also be involved in maintaining the steady-state strabismus. The possible involvement of such structures ranges from altered eye muscle lengths to neural mechanisms that alter eye muscle tone or contractility. Our research has therefore been directed towards identifying and understanding the roles of specific areas in the brain that may be involved in producing oculomotor properties describing the strabismus state. To this end, we have been using a multi-pronged strategy involving behavioral studies of eye alignment, eye movements and ocular accommodation and single cell recording studies of information processing in neural oculomotor circuits. I have the expertise and background necessary to carry out this research. I am broadly trained as a bioengineer (PhD) and as an oculomotor neurophysiologist (post-doc). During my post-doctoral fellowship at the Yerkes Primate Center, I first became interested in examining neural oculomotor mechanisms in non-human primate models for strabismus. This translated into independent investigator funding (R01 EY015312) from the NIH in 2004. I moved my laboratory and my funding to the College of Optometry, University of Houston in 2009 and have since continued the same line of research. In 2012, I received additional funding (R01-EY022723) to examine neural adaptive responses that may occur as a result of conventional surgical treatment of strabismus in monkeys. This project has direct implications for translation to human treatment methods. In the current application, I am proposing to investigate the contribution of the Superior Colliculus in three critical oculomotor disruptions associated with strabismus – Eye Misalignment, Fixation Instability and Fixation-Switch.

1. Das VE. Responses of cells in the midbrain near-response area in monkeys with strabismus. Invest Ophthalmol Vis Sci 53(7); 3858-3864; June 2012.
2. Joshi AC, Das VE. Muscimol Inactivation of the Caudal Fastigial Nucleus and Posterior Interposed Nucleus in Monkeys with Strabismus. J. Neurophysiology. 110(8); 1882-91; Oct 2013. PMCID:

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PMC3798947.
3. Agaoglu MN, Kim SL, Joshi AC, Das VE. Spatial Patterns of Fixation-Switch Behavior in Monkeys with Strabismus. Invest Ophthalmol Vis Sci 55(3) 1259-68; March 2014. PMCID: PMC3943416.
4. Tusa RJ, Mustari MJ, Das VE, Boothe RG. "Animal models for visual deprivation-induced strabismus and nystagmus." Annals of the New York Academy of Sciences; 956: 346-60, 2002.

B. Positions and Honors

Positions and Employment

2000 - 2001	Research Associate, Division of Visual Sciences, Yerkes Regional Primate Research Center, Emory University, Atlanta, GA.
2002 - 2003	Instructor, Department of Neurology, Emory University, Atlanta, GA.
2003 - 2009	Assistant Professor, Department of Neurology, Emory University, Atlanta, GA
2003 - 2009	Research Assistant Professor, Yerkes National Primate Research Center, Emory University, Atlanta, GA.
2004 - 2009	Assistant Professor, Neuroscience Program, Graduate Division of Biological & Biomedical Sciences, Emory University, Atlanta, GA.
2006 - 2009	Core-staff, Yerkes National Primate Research Center, Emory University, Atlanta, GA
2009 – Present	Associate Professor, College of Optometry, University of Houston & Department of Biomedical Engineering, University of Houston
2014 – Present	Professor, College of Optometry, University of Houston & Department of Biomedical Engineering, University of Houston

Other Experience and Professional Memberships

1999 – Present	Member, Society for Neuroscience
2001 - Present	Member, Association for Research in Vision and Opthalmology (ARVO)
2004 - 2009	Member, Institutional Animal Care and Use Committee, Emory University
2007 – Present	Editorial Board of the journal 'Strabismus'
2009 – Present	Member, Institutional Animal Care and Use Committee, University of Houston
2014-Present	Member, Faculty Senate, University of Houston

Honors

- NIH NRSA Fellowship through Dept of Neurology, Emory University.
- Ad-Hoc Reviewer, NIH/CSR - Anterior Eye Study Section, 2006
- Review panel member, NIH/CSR – NEI K99 Study Section, 2007
- Ad-Hoc Reviewer, NIH/CSR – ARRA Mail-in Reviewer, 2009
- Ad-Hoc Member, CSR, NIH – Special Emphasis Panel (ZRG1 SBIB-V (82) S); November 2013 & 2014
- Invited to organize ISER 2012 strabismus symposium, Berlin, Germany (2012) – "Understanding central peripheral and genetic changes in strabismus: Disruptions in neural circuits in monkeys with strabismus."
- Invited speaker at Gordon Research Conference on Eye Movements in 2011 and 2015. (2015 Meeting Presentation Title: Disruption in Vergence Neural Circuits as a Mechanism for Developmental Strabismus)
- ARVO Annual Meeting Program Committee Member (EY section; Term: 2012-2015; Chair in 2015)

C. Contribution to Science

1) My early publications as a graduate student working under the mentorship of Dr. John Leigh at Case Western Reserve University covered a wide area of topics in the oculomotor system including studies in both normal individuals and patients with neurological disease. A major focus was on visual-vestibular interactions, specifically examining the nature of interactions at high frequencies of head rotation, at which the vestibular system functions efficiently but the visual system does not. I

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gained an appreciation of working with disease conditions at this time and this experience has helped during my current studies examining strabismus mechanisms.

a. Das VE, Zivotofsky AZ, DiScenna AO, Leigh RJ: Head perturbations during walking while viewing a head-fixed target. Aviation, Space and Environmental Medicine 66: 728-732, 1995.
b. Das VE, Leigh RJ, Thomas CW, Averbuch-Heller L, Zivotofsky AZ, DiScenna AO, Dell'Osso LF. Modulation of high-frequency vestibulo-ocular reflex during visual tracking in humans. J Neurophysiol 74: 624-632, 1995.
c. Das VE, DiScenna AO, Feltz A, Yaniglos SS, Leigh RJ. Tests of a linear model of visual-vestibular interaction using the technique of parameter estimation. Biol Cybern; 78, 183-195, 1998.
d. Das VE, Dell'Osso LF, Leigh RJ. Enhancement of the vestibulo-ocular reflex by prior eye movements. J. Neurophysiology. 81: 2884-2892, 1999.
e. Das VE, Oruganti P, Kramer PD, Leigh RJ. Experimental tests of a neural network model for ocular oscillations caused by diseases of central myelin. Experimental Brain Research. 133:189-197, 2000.

2) Building upon the previous work, I collaborated on a series of studies examining visual and vestibular processing and gaze control in normal monkeys. We used a modeling approach to identify the encoding of retinal error and eye movement signals in different brain areas (NOT, DLPN and NRTP) the relative contributions of position, velocity and acceleration signals to the neuronal response rates.

a. Das VE, Economides JR, Ono S, Mustari MJ. Information Processing By Parafoveal Cells In The Primate Nucleus Of The Optic Tract. Experimental Brain Research 140: 301-310, 2001.
b. Ono S, Das VE, Mustari MJ. Role of the Dorsolateral Pontine Nucleus In Short-Term Adaptation Of The Gain Of The Horizontal Vestibulo-Ocular Reflex. J Neurophysiology 89(5): 2879-85, 2003.
c. Ono S, Das VE, Mustari MJ. Gaze related response properties of DLPN and NRTP Neurons in the Rhesus Macaque J Neurophysiology 91: 2484-2500, 2004.
d. Ono S, Das VE, Economides JR, Mustari MJ. Modeling of Smooth Pursuit related neuronal responses in the DLPN and NRTP of the rhesus macaque. Journal of Neurophysiology. 93(1): 108-16, 2005 Jan.

3) Establishing a non-human primate model for developmental sensory strabismus has been an important focus. We have done several studies establishing various behavioral properties of strabismic animals that have shown that the strabismus model represents the human condition. The properties that we have described in our animal model include horizontal misalignment, A/V patterns, Dissociated horizontal and vertical deviations, latent nystagmus, saccade disconjugacy and alternating fixation. Some of these studies have formed the basis of the neurophysiology studies examining disruption of neural circuits in the strabismic animal.

a. Tusa RJ, Mustari MJ, Das VE, Boothe RG. "Animal models for visual deprivation-induced strabismus and nystagmus." Annals of the New York Academy of Sciences; 956: 346-60, 2002.
b. Das VE, Ono S, Tusa RJ, Mustari MJ. Conjugate Adaptation of Saccadic Gain in Non-Human Primates with Strabismus. J Neurophysiology 91: 1078-1084, 2004.
c. Das VE, Fu LN, Mustari MJ, Tusa RJ. Incomitance in monkeys with strabismus. Strabismus 13: 33-41, 2005.
d. Fu LN, Tusa RJ, Mustari MJ, Das VE. Horizontal saccadic disconjugacy in monkeys with strabismus. Invest Ophthalmol Vis Sci. 48(7): 3107-14; Jul 2007.
e. Das VE. "Investigating mechanisms of Strabismus in nonhuman primates". J. AAPOS. 2008 Aug;12(4):324-5. PMCID: PMC2601707

4) We are one of very few labs that have undertaken the investigation of neural oculomotor circuits in monkeys with strabismus. We were the first to establish that the brain is involved on a moment-by-moment basis in setting the steady-state strabismus angle. We then went to establish that a 'vergence' circuit (Cerebellum-Supraoculomotor area-motor nucleus) is implicated in maintaining

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the steady state of strabismus. Aim 1 of the current application will examine the role of the rostral Superior Colliculus in setting eye misalignment. During one of our studies, we serendipitously induced hemi-seesaw nystagmus (HSSN) thus shedding light on a debate on the neural mechanism driving HSSN. We concluded that inactivating the caudal aspect of the interstitial nucleus of Cajal leads to HSSN.

a. Das VE, Mustari MJ. Correlation of cross-axis eye movements and motoneuron activity in non-human primates with "A" pattern strabismus. Invest Ophthalmol Vis Sci. 48(2):665-74; Feb 2007.
b. Joshi AC, Das VE. Responses of medial rectus motoneurons in monkeys with strabismus. Invest Ophthalmol Vis Sci 52(9); 6697-6705; Aug 2011.
c. Das VE. Responses of cells in the midbrain near-response area in monkeys with strabismus. Invest Ophthalmol Vis Sci 53(7); 3858-3864; June 2012.
d. Joshi AC, Das VE. Muscimol Inactivation of the Caudal Fastigial Nucleus and Posterior Interposed Nucleus in Monkeys with Strabismus. J. Neurophysiology. 110(8); 1882-91; Oct 2013. PMCID: PMC3798947.
e. Das VE, Leigh RJ, Swann M, Thurtell MJ. "Muscimol inactivation caudal to the interstitial nucleus of cajal indues hemi-seesaw nystagmus". Exp Brain Research. 2010 Sep: 205(3) 405-13. PMCID: PMC2965773.

5) A major recent focus has been to examine fixation switch behavior in monkeys with strabismus. This phenomenon is important and interesting because it is the oculomotor aspect of viewing objects in the environment in the presence of visual suppression. This project also has relevance to understanding how the oculomotor might process information from each eye (in the presence of visual suppression) in order to generate a saccade that brings one or the other eye onto the target. Aim 3 of the current application will examine the role of the Superior Colliculus in driving this behavior.

a. Das VE. "Alternating Fixation and Saccade Behavior in Non-Human Primates with Alternating Occlusion Induced Exotropia" Invest Ophthalmol Vis Sci 2009 Aug; 50(8) 3703-3710. PMCID: PMC2837805.
b. Agaoglu MN, Kim SL, Joshi AC, Das VE. Spatial Patterns of Fixation-Switch Behavior in Monkeys with Strabismus. Invest Ophthalmol Vis Sci 55(3) 1259-68; March 2014. PMCID: PMC3943416.
c. Agaoglu S, Agaoglu MN, Das VE. Motion Information via the Non-Fixating Eye can drive Optokinetic Nystagmus in Strabismus (Invest Ophthalmol Vis Sci – under revision 2015).

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/vallabh.das.1/bibliography/40830031/public/?sort=date&direction=ascending

D. Research Support

Ongoing Research Support

NIH (NEI) R01 EY022723 Das (PI) Dates: 08/01/2012 – 07/31/2016
"Neural Adaptation to Strabismus Surgery"
Experiments associated with this grant attempt to elucidate the neural adaptive response that may occur as a result of conventional surgical treatment of strabismus. It is hypothesized that in cases where the strabismus correction surgery fails (strabismus returns), the neural drive from the brain has adapted to counter the mechanical effects of surgery. To test this hypothesis, we will record from a population of motoneurons in the oculomotor and abducens nuclei in juvenile monkeys with strabismus before and after surgical correction of the strabismus. There is no overlap with the current application. Monkeys cannot be shared between the two proposals, because in the current grant application we will examine animals that have not any intervention (as in strabismus correction surgery) applied.
Role: Principal Investigator

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NIH (NEI) P30 EY007751 (PI: Frishman) Dates: 1997 – 2018
"Core Grant for Vision Research"
This NEI Core Grant to the College of Optometry supports shared research modules for computer programming, biostatistics, instrument design, bioimaging and fabrication. As a faculty member with an active NIH R01 grant, I am listed as an investigator on the grant.
Role: Core Investigator

Completed Research Support

NIH (NEI) R01 EY01532 Das (PI) Dates: 02/01/2004 – 07/30/2014
"Binocular Coordination of Eye Movements"
Studies associated with this grant examined the role of the supraoculomotor area and the deep cerebellar nuclei (fastigial nucleus and posterior interposed nucleus) in determining the state of strabismus including horizontal misalignment, 'A/V' patterns of strabismus and associated abnormal eye movements in juvenile animals with strabismus.
Role: Principal Investigator

NIH (NEI) Mustari (PI) Dates: 06/07/2002-01/31/2011
"Neural control of visual-vestibular behavior"
Assessed the role of the MST-DLPN and FEF-NRTP pathways in normal visual-vestibular function and gaze control.
Role: Co-Investigator

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PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix: Dr.
First Name*: Vallabh
Middle Name: Easwara
Last Name*: Das
Suffix:

2. Human Subjects

Clinical Trial? ○ No ○ Yes
Agency-Defined Phase III Clinical Trial?* ○ No ○ Yes

3. Permission Statement*

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

● Yes ○ No

4. Program Income*

Is program income anticipated during the periods for which the grant support is requested? ○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

Budget Period*	Anticipated Amount ($)*	Source(s)*

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5. Human Embryonic Stem Cells

Does the proposed project involve human embryonic stem cells?* ● No ○ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): Specific stem cell line cannot be referenced at this time. One from the registry will be used.

6. Inventions and Patents (For renewal applications only)

Inventions and Patents*: ○ Yes ○ No

If the answer is "Yes" then please answer the following:

Previously Reported*: ○ Yes ○ No

7. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:

First Name*:

Middle Name:

Last Name*:

Suffix:

❑ Change of Grantee Institution

Name of former institution*:

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PHS 398 Modular Budget

OMB Number: 0925-0001

Budget Period: 1

Start Date: 04/01/2016 End Date: 03/31/2017

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium F&A*	250,000.00
Consortium F&A	0.00
Total Direct Costs*	250,000.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
1.	MTDC_On Campus FY2015	50.50	250,000.00	126,250.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Arif Karim, 214-767-3261

Indirect Cost Rate Agreement Date: 08/24/2011

Total Indirect Costs: 126,250.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($): 376,250.00

Funding Opportunity Number: PA-13-302. Received Date: 2015-06-03T14:39:23.000-04:00

PHS 398 Modular Budget

Budget Period: 2

Start Date: 04/01/2017 End Date: 03/31/2018

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium F&A*	250,000.00
Consortium F&A	0.00
Total Direct Costs*	250,000.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
1.	MTDC_On Campus FY2015	50.50	250,000.00	126,250.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Arif Karim, 214-767-3261

Indirect Cost Rate Agreement Date: 08/24/2011

Total Indirect Costs: 126,250.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($): 376,250.00

Funding Opportunity Number: PA-13-302. Received Date: 2015-06-03T14:39:23.000-04:00

PHS 398 Modular Budget

Budget Period: 3

Start Date: 04/01/2018 End Date: 03/31/2019

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium F&A*	250,000.00
Consortium F&A	0.00
Total Direct Costs*	250,000.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
1.	MTDC_On Campus FY2015	50.50	250,000.00	126,250.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Arif Karim, 214-767-3261

Indirect Cost Rate Agreement Date: 08/24/2011

Total Indirect Costs: 126,250.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($): 376,250.00

PHS 398 Modular Budget

Budget Period: 4

Start Date: 04/01/2019 End Date: 03/31/2020

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium F&A*	250,000.00
Consortium F&A	0.00
Total Direct Costs*	250,000.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
1.	MTDC_On Campus FY2015	50.50	250,000.00	126,250.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Arif Karim, 214-767-3261

Indirect Cost Rate Agreement Date: 08/24/2011

Total Indirect Costs: 126,250.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($): 376,250.00

PHS 398 Modular Budget

Budget Period: 5	
Start Date: 04/01/2020 End Date: 03/31/2021	

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium F&A*	250,000.00
Consortium F&A	0.00
Total Direct Costs*	250,000.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
1.	MTDC_On Campus FY2015	50.50	250,000.00	126,250.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Arif Karim, 214-767-3261

Indirect Cost Rate Agreement Date: 08/24/2011

Total Indirect Costs: 126,250.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($): 376,250.00

PHS 398 Modular Budget

Cumulative Budget Information

1. Total Costs, Entire Project Period

Section A, Total Direct Cost less Consortium F&A for Entire Project Period ($)	1,250,000.00
Section A, Total Consortium F&A for Entire Project Period ($)	0.00
Section A, Total Direct Costs for Entire Project Period ($)	1,250,000.00
Section B, Total Indirect Costs for Entire Project Period ($)	631,250.00
Section C, Total Direct and Indirect Costs (A+B) for Entire Project Period ($)	1,881,250.00

2. Budget Justifications

Personnel Justification	Das_StrabismusSC_BudgetJustification_Personnel1011854067.pdf
Consortium Justification	
Additional Narrative Justification	

Funding Opportunity Number: PA-13-302. Received Date: 2015-06-03T14:39:23.000-04:00

BUDGET JUSTIFICATION

PERSONNEL

Principal Investigator (Vallabh E. Das PhD): Salary support [redacted] person-months) is requested for Dr. Das who will be responsible for all phases of the project. This includes prism rearing to induce strabismus and all experimental studies in juvenile strabismic monkeys. The PI is also responsible for all phases of data analysis and publication of results.

Postdoctoral fellow: Salary support (12 person-months) is requested for a post-doctoral fellow who will play a major role in the behavior and physiology experiments. This position will be filled once we have the funding to do so.

Graduate Student: Salary support (12 person-months) is requested for a graduate student who will play a major role in the behavior and physiology experiments. Ms. [redacted], a graduate student in the College of Optometry, University of Houston is currently holding this position.

Laboratory Supervisor: Salary support (12 person-months) is requested for a lab supervisor/research technician. The major responsibilities will include animal rearing, animal care management and general laboratory management such as manufacturing electrodes, ordering supplies, etc. The laboratory supervisor is essential to the successful completion of the proposed studies. [redacted] who joined the lab in 2014 is currently holding this position.

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PHS 398 Research Plan

Please attach applicable sections of the research plan, below.

OMB Number: 0925-0001

1. Introduction to Application (for RESUBMISSION or REVISION only)	
2. Specific Aims	Das_StrabismusSC_GrantFinal_Specific_Aims1011854241.pdf
3. Research Strategy*	Das_StrabismusSC_GrantFinal_Research_Strategy1011854242.pdf
4. Progress Report Publication List	
Human Subjects Sections	
5. Protection of Human Subjects	
6. Inclusion of Women and Minorities	
7. Inclusion of Children	
Other Research Plan Sections	
8. Vertebrate Animals	Das_StrabismusSC_Vertebrate_Animals1011854068.pdf
9. Select Agent Research	
10. Multiple PD/PI Leadership Plan	
11. Consortium/Contractual Arrangements	
12. Letters of Support	
13. Resource Sharing Plan(s)	
Appendix (if applicable)	
14. Appendix	

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Section A: Specific Aims

Strabismus (exotropia – divergent strabismus; esotropia – convergent strabismus) is most commonly a developmental disorder that affects approximately 2-5% of all human infants[1, 2]. The long-term goal in this project is to understand oculomotor mechanisms underlying disruption in binocular alignment and binocular coordination of eye movements in strabismus. Our strategy is to utilize a basic science approach with behavioral studies of eye alignment and eye movements, and neurophysiological studies of oculomotor circuits in awake-behaving strabismic monkeys. We have previously conducted studies that showed the role of cerebellar and midbrain areas in partially establishing the steady state of strabismus, including properties such as A/V patterns and Dissociated Horizontal and Vertical Deviations. We now propose to investigate the neural basis for three strabismus properties – 1) eye misalignment, 2) fixation instability and 3) fixation-switch. Each of these strabismus properties implicates an important oculomotor control structure, the Superior Colliculus (SC), and our plan is to couple behavioral studies with neurophysiological investigation (single cell recording and muscimol inactivation studies) of SC contribution. Studies will be performed in juvenile (3-4 years old) rhesus monkeys previously induced with a sensory form of strabismus by rearing them under special viewing conditions (prism-rearing) for the first four months of their life.

Aim 1: To investigate the role of the rostral SC (rSC) in driving the state of eye misalignment

We previously established that a vergence eye movement circuit involving projections from cerebellar nuclei (Fastigial Nucleus and Posterior Interposed Nucleus) to the midbrain supraoculomotor area (SOA) and thereafter to the oculomotor nucleus (OMN) and medial rectus (MR) muscle is partially responsible for maintaining horizontal eye deviation in strabismus. Studies in normal monkeys have also implicated the superior colliculus in vergence including via a recent finding of convergence and divergence neurons in the rSC. One study offered a framework wherein projections from the rSC to the abducens nucleus via the central mesencephalic reticular formation could complement the cerebellum->SOA->OMN pathway for slow vergence movements. Therefore we propose single unit recording and muscimol inactivation studies of the strabismic monkey rSC to establish its role in strabismus. *Hypothesis: The rSC encodes a static bias for ocular misalignment in strabismic monkeys. Prediction: We expect to identify encoding of strabismus angle in rSC 'vergence' cells. Population vergence (eye misalignment) sensitivity of these cells will differ from normal monkeys providing a basis for altered extraocular muscle tone and therefore strabismus. Muscimol inactivation of rSC will eliminate the static bias resulting in a modification of strabismus angle.*

Aim 2: To investigate the role of the rostral SC in driving fixation instability

Studies in strabismic and amblyopic humans and monkeys have identified increased fixation instability that is driven by large drifts and nystagmus during attempted fixation. According to current theory in normal monkeys, optimal fixation location and microsaccade behavior is governed by balanced activity across the two rSC. Here we will investigate fixation instability in strabismic monkeys and establish (via single unit recording and muscimol inactivation) how the rSC contributes to fixation instability in these animals. *Hypothesis: The rSC contributes to fixation instability in strabismic monkeys by driving quick phases of nystagmus. Prediction: rSC "fixation cells" will show correlated activity to nystagmus quick phases (in addition to microsaccades) but not slow phases and drift during fixation. Muscimol inactivation of the rSC will result in an overall decrease in the number of quick phases. Since quick phases act to reset eye position following drift, overall fixation instability will increase following rSC inactivation.*

Aim 3: To investigate the role of the SC in driving fixation-switch behavior

Under binocular viewing, some strabismic patients develop the ability to change their eye of fixation depending upon spatial target location (e.g., right eye fixating prior to target step and left eye fixating at end of saccade – referred to as fixation-switch). *Hypothesis: We propose a framework wherein, although only one target is perceived (visual suppression prevents double vision during binocular viewing), the oculomotor system has access to retinal error signals from both the fixating and deviated eyes. Thereafter, fixation-switch is achieved via a competitive decision process in which the brain chooses between these two errors to program a saccade that leads to one of the eyes foveating the target.* In a general sense, this hypothetical framework is equivalent to that of a normal oculomotor system selecting between two simultaneously presented targets. We will test our hypothesis for a fixation-switch mechanism by recording from caudal SC since this structure plays an important role in target selection and spatial attention. *Prediction: Visual, build-up and burst activity of neurons in the Superior Colliculus of strabismic monkeys will show activity during fixation-switch that is similar to activity observed in such cells during target selection tasks in normal monkeys.*

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Section B: Significance

Binocular alignment and binocular coordination of eye movements are important in foveate species[3, 4]. Developmental loss of sensory or motor fusion leads to ocular misalignment (strabismus) in as much as 5% of all children making this disease a significant public health issue[2, 4]. The exact cause of strabismus is unknown[5] [6]. Many diverse factors including refractive errors (anisometropia), visual acuity factors (congenital cataracts), genetic factors (Congenital Fibrosis of extraocular muscles (EOM), Marfan's syndrome), brainstem pathology (Duane's syndrome) and muscle pathology (Dysthyroid opthalmopathy), likely trigger a cascade of events that result in misaligned eyes. The common thread among the sensory causes for strabismus appears to be a disruption of binocular vision during the early critical period for development that hypothetically leads to disruptions in visual and oculomotor areas and eventually misaligned eyes. In other words, developmental strabismus usually begins as a 'brain' problem. However, investigations of strabismus found in the literature are primarily oriented toward evaluating outcomes of surgical intervention. *Our approach is significant and also innovative because we are proposing to improve basic understanding of strabismus mechanisms by performing neurophysiological studies in non-human primate models.* Our previous studies were the first to investigate neural involvement by identifying strabismus related encoding in cerebellar and midbrain areas.

Aim 1 is significant because we will investigate an important oculomotor structure (rSC), potentially unraveling additional components of a slow vergence circuit that also drives eye misalignment. Our studies are also significant to the discussion in the oculomotor community regarding Hering (binocular) and Helmholtz (monocular) frameworks for binocular control. In an attempt to reconcile conflicting views, an amalgamated framework for binocular eye movements was proposed[7] where a distinction was made between 'fast' and 'slow' vergence. 'Fast' or 'saccadic' vergence is driven via monocular circuits (e.g.: as in the PPRF[8]) and 'slow' vergence, necessary for vergence pursuit, fine tuning of binocular position after the fast component and presumably static alignment, is driven by binocular control areas (e.g.: midbrain SOA neurons[9]). Our investigation thus far in strabismic monkeys supports this new framework - SOA recording[10], cerebellar muscimol studies[11] and preliminary data from rSC show that a static strabismus signal is encoded within the 'slow vergence' circuit. In strabismic monkeys, disconjugate eye movements observed during saccades[12], could be due to disruption in the monocular control of 'fast' vergence. Therefore, adding significance to our studies, we plan to analyze ocular specificity (i.e., is firing rate better correlated with one of the eyes or a conjugate signal?) in SC neurons during saccadic movements (Aims 2 and 3) thereby testing the different frameworks; aiding our analysis is the presence of disconjugate saccades[12] and inappropriate cross-axis components due to A/V patterns[13].

Fixation instability is a significant problem associated with strabismus and amblyopia and could be partly responsible for the reduction of visual acuity associated with the disease. Little is known about neural mechanisms that are the basis for fixation instability in strabismus/amblyopia. Our experiments (aim 2) are significant because they will, for the first time, directly assess the role of an oculomotor structure (rSC) in determining fixation instability in disease. Another significant contribution is that these studies will be the first to correlate rSC neuronal activity and the quick-phases of pathological nystagmus (previous reports have only examined rSC neuronal activity during physiological nystagmus like optokinetic and vestibular nystagmus).

Aim 3 investigates the neural basis for the spatial patterns of target acquisition behavior (including fixation-switch) in the strabismic monkey. Examining fixation-switch behavior in strabismus is significant because it is the oculomotor aspect of attempting to acquire and fixate targets in the presence of visual suppression. Beyond its significance to understanding strabismus mechanisms, studying fixation-switch addresses a question significant to all neuroscience - How is sensation (visual information via the fixating and deviated eyes) converted into action (a saccade that leads to fixation with either eye) in strabismus?

Innovation

In general, a neural systems approach to the study of motor aspects of strabismus is lacking. The fundamental innovation in our project lies in combining appropriate animal models, a strong conceptual framework based on binocular coordination in normals, and established neurophysiological techniques to directly study a clinical problem (strabismus). Additionally, these studies are focused in a brain area that has been extensively studied in normal animals but is yet to be approached in disease models. As a consequence we will also be testing frameworks developed in normal monkeys (e.g., control of fixation by rSC) by applying these frameworks in our strabismus model. Technical innovations, which will increase data yield and improve precision, are use of an injectrode for muscimol experiments (Aims 1, 2), simultaneous dual electrode recording (Aim 3) and use of an automated method (similar to image stabilization) for improving precision of presenting targets in the neuron's visual receptive field (Aim 3).

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Approach

Aim 1: To investigate the role of the rostral SC in driving the state of eye misalignment

The literature often describes strabismus in terms of "underaction" or "overaction" of EOM. However this is a description of state and not of mechanism and neural signals in oculomotor structures likely plays a critical role in determining the state of strabismus[14, 15]. Here we will test the role of the rSC in driving misalignment.

Significance, Justification and Feasibility

Disruption of a vergence circuit as a mechanism for strabismus

We have previously recorded from medial rectus motoneurons (MRMN) in the oculomotor nucleus (OMN) of strabismic monkeys and showed that activity was directly correlated with the position of the deviated eye (for both horizontal misalignment and A/V patterns) lending strong support to the idea that the brain is intimately involved in determining the strabismus state on a moment-to-moment basis[16]. Abducens neurons show similar response properties as MRMN indicating that medial and lateral recti act in push-pull to establish the strabismic state[17, 18]. *It is likely that central neural structures play a critical role in determining the state of strabismus and we previously identified neurons in the supraoculomotor area (SOA-projects monosynaptically to MRMN) that encode the horizontal strabismus angle*[10]. These neurons, which encode vergence eye movements in normal monkeys, show altered response characteristics in strabismics (lower slopes and shifted thresholds compared to normal) resulting in the modification of the 'vergence' tone of MR muscles and therefore providing a neural basis for misalignment. Interestingly these neurons did not encode changes in strabismus angle due to A/V patterns. Our most recent study examined the role of cerebellar structures (caudal fastigial nucleus - cFN, and posterior interposed nucleus - PIN) that project to the SOA[11]. We found that pharmacological inactivation of the cFN using muscimol produced a divergent change in eye misalignment (a reduction of esotropia and an increase in exotropia) while inactivation of the PIN produced a convergent change in eye misalignment. Thus the cFN and PIN exert significant and complementary influences on steady-state strabismus angle. *Based on the above series of studies, we published a framework wherein normal alignment is achieved by overall balanced activity across convergence and divergence cells in a 'slow' vergence pathway. Developmental disruption, in the form of a static bias, within this pathway is responsible for setting the state of static misalignment*[11]. *One arm of this circuit involves the cFN/PIN -> SOA -> MRMN -> MR muscle pathway (Figure 1). A complementary yet unidentified pathway likely provides a strabismus signal to the LR muscle. The goal of the proposed experiments is to determine the role of the rostral SC in eye misalignment in strabismic monkeys. Our working hypothesis is that the rSC is encoding a static bias for ocular misalignment.*

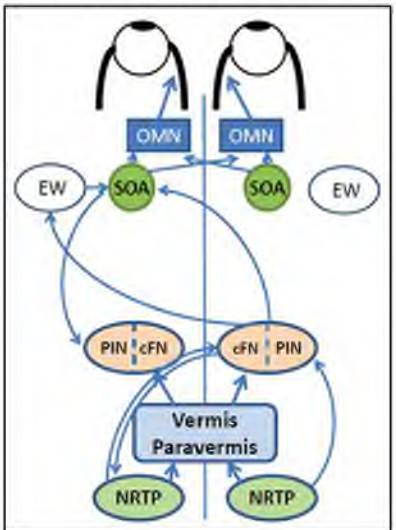

Fig 1. Hypothetical vergence pathway that provides strabismus drive to the MR muscle. NRTP – Nucleus Reticularis Tegmenti Pontis; EW – Edinger-Westphal nuc.

Evidence implicating the Superior Colliculus in vergence and strabismus

Relative to the work on SC role in saccades, there are only a few studies on SC role in vergence. Jiang et al identified neurons in the cat rSC related to convergence[19]. Also, electrical stimulation or pharmacological inactivation of the cat rSC produced changes in both accommodation and vergence[20-22]. Monkey studies also suggest a role of the SC in vergence, though perhaps interpretation is more complex. Billitz and Mays were not able to elicit vergence by electrical stimulation (ES) in the rSC during far viewing but ES during near viewing caused a relaxation of vergence[23]. In another study, ES in rSC interfered with vergence only if applied just before or during a vergence only movement or a combined saccade-vergence movement[24, 25]. One hypothesis for the apparent lack of vergence changes due to ES in normal monkeys is that a net 'zero vergence' command is initiated because both convergence and divergence related neurons are activated. Lawler and Cowey performed ablations of the rSC in monkey and suggested that there were problems with both disparity processing and eye alignment although eye movements were not explicity recorded in this study[26]. In another study, neurons in the SC were shown to receive monosynaptic projections from cortical LIP neurons that also encoded depth information[27]. Walton and Mays showed that saccade related neurons in the caudal colliculus showed a weak relationship to vergence in that many burst neurons showed a reduction in saccade velocity sensitivity when looking at near targets compared to when looking at far targets[28]. However they were unable to identify any systematic 3-D tuning of neurons. *Interestingly, two studies investigating SOA vergence neurons*

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also reported another population of vergence neurons located 4-5mm dorsal and 2-3mm lateral to the OMN that they did not unequivocally localize using histological methods, but suggested could be in the rSC[29, 30]. A recent study by Van Horn et al (2013) identified convergence and divergence neurons in the rSC that were modulated during slow vergence but not conjugate or fast vergence eye movements[31], thereby postulating that the rSC only contributes to slow vergence. Also, ES in this area produced vergence angle changes.

So how might the rSC be involved in strabismus? Ohtsuka reported a patient with a focal lesion in the rostral SC, who showed a deficit in convergence and a static exotropia[32]. The afferent and efferent anatomical projections of the SC are diverse[33]; focusing on potential 'vergence' pathways, the SC receives extensive projections from cortical disparity areas including LIP and FEF. It also receives projections from the cFN and the PIN[34], areas that we showed is related to the strabismus state[11]. A major efferent target of the SC is the cMRF (lateral to OMN) and studies have identified monosynaptic connections between the cMRF and the abducens nucleus[35, 36]. We hypothesize that developmental disruption of binocular vision leads to a static bias in the rSC (i.e., not net zero vergence encoding but a signal for static misalignment). Zhang et al antidromically activated SOA neurons (but presumably not SC vergence neurons) from the MRMN's suggesting that the efferent projection of SOA and SC vergence neurons could differ[37, 38]. Therefore the SC->cMRF->abducens nucleus pathway could provide strabismus drive to the lateral rectus. *Supporting our hypothesis of rSC involvement in strabismus, in preliminary investigation, we have found cells that show activity correlated to strabismus angle (Fig 5, 9); also electrical stimulation (Fig 4) produced changes in strabismus angle.*

Animal models for strabismus

Surgical or sensory approaches may be used for creating animal models of strabismus. Surgical methods induce strabismus by disrupting motor fusion, e.g., muscle tenotomy[39-44]. Sensory methods induce strabismus by rearing infants under special viewing conditions. Disrupting binocular vision in the first months of life in a monkey is sufficient to produce strabismus[45-47]. *We have significant experience inducing strabismus using sensory methods (see our publications). For the proposed experiments, we will use an optical prism rearing method for the first four months of life (starting from day 1 after birth) to induce strabismus.*

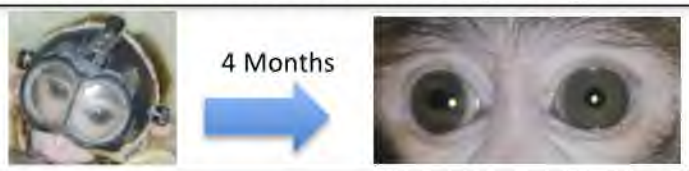

Fig 2. Optical Prism rearing of infant monkeys for first 4 months of life starting from day 1 after birth results in a permanent strabismus. Left Panel: Infant monkey viewing through prisms. Right panel: Same monkey at ~6 months of age

Prism strabismus or optical strabismus[48, 49], is based on the premise that the visual axes are separated due to prism viewing (decorrelated binocular visual input) to an extent that sensory fusion cannot be achieved during development[50, 51]. Viewing through prisms for the first three months of life in a monkey can induce a permanent esotropia[52] or exotropia[53]. There is profound loss of binocular neurons in V1/V2 of prism-reared animals[54, 55], similar to the finding in an alternating monocular occlusion model for strabismus[45]. Studies in prism-reared animals have described strabismus properties such as Latent Nystagmus, Naso-temporal asymmetry, Dissociated Deviations, and A/V patterns in both esotropic and exotropic animals[52, 56-59]. *Many of our previous studies used a daily alternating monocular occlusion (AMO) rearing paradigm to induce strabismus. Recently we have been using the prism-rearing paradigm[17, 58-60] and the oculomotor and neural disruptions that we are testing here (misalignment, fixation instability and fixation-switch) are well replicated in both models (Fig 3). An advantage of the prism model is that it introduces binocular de-correlation during development that leads to strabismus and therefore may be more representative of human strabismus.*

Preliminary Studies

Preliminary data in all aims illustrate salient eye movement properties in the strabismic monkeys and show technical capability in conducting the proposed experiments. Fig 3 shows eye misalignment in strabismic monkeys reared under either AMO (Fig 3A) or prism-viewing (Fig 3B). Electrical stimulation (ES) is commonly used to map the SC. ES in the SC results in a staircase of contralateral saccades whose vector depends on stimulation location[61]; rostral sites elicit smaller saccades. Fig 4, from Prism-reared monkey M2, shows ES (left SC; 400Hz, 20μamp, 0.5s duration; multiple trials aligned on ES onset) that led to rightward and downward saccades of ~5° radial amplitude. *ES at this site also induced a divergent change in misalignment (exotropia, cyan trace, is increased during ES). There is minimal change in vertical vergence.*

Many strabismic animals show a change in misalignment based on which eye is viewing the target (dissociated horizontal deviation-DHD). We previously leveraged this observation to identify strabismus angle

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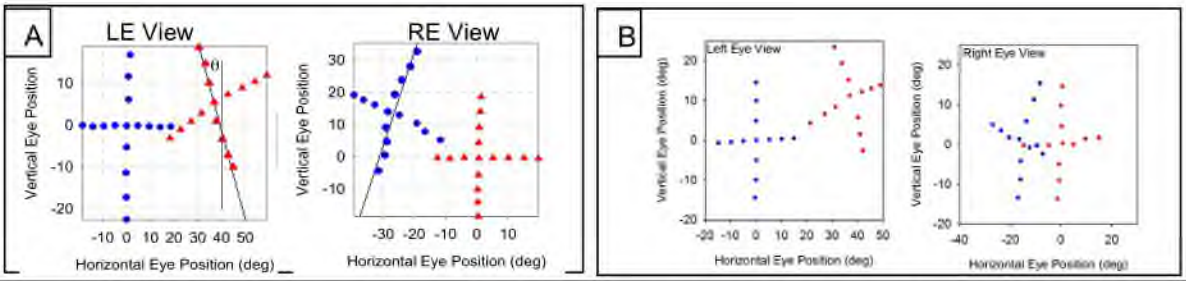

Fig 3: Panel A: Exotropia, A-pattern strabismus, Dissociated Vertical and Horizontal Deviations revealed during monocular fixation on targets along the horizontal or vertical meridian in an AMO strabismic monkey (from Das et al 2005). +ve values indicate rightward or upward eye positions in all figures; -ve values show leftward or downward positions. Panel B: Similar misalignment properties are observed in a strabismic monkey reared using optical prism-viewing. Legend: Right Eye – red; Left Eye - blue.

cells in the SOA[10]. *We now propose studying the rSC to identify another population of strabismus related cells.* Fig 5 shows two cells that both show a relationship to eye misalignment - One cell was recorded in the SOA (Monkey M1, Panels A-D; Das 2012) and one in the rSC (Monkey M2, Panels E-H; cell location - 1mm rostral from and similar depth as Fig4 ES site). The example SOA cell shows greater firing at smaller angles of exotropia (near-response cell) while the example rSC cell shows greater firing for a larger angle of exotropia (far-response cell). Both cell types were found in the SOA and the same may be true in the rSC. The inset in panel E shows a magnified view of a couple of trials and illustrates the ongoing nystagmus when the animal was viewing with the left eye. Since the nystagmus is small, it does not interfere with analysis of misalignment related firing. In Aim 2, we ask whether these fixation/misalignment cells also encode nystagmus quick phases [62, 63]. Preliminary data (Fig 9) suggests that it does. *We also propose inactivation studies to determine the role of the rSC in setting eye misalignment (Aim 1) or fixation stability (Aim 2).* We have experience with injecting muscimol in various brain regions[11, 64, 65] and Fig 6 shows a divergent change in strabismus angle following rSC inactivation.

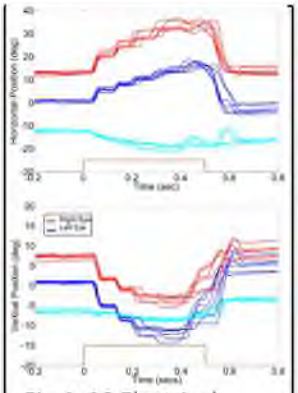

Fig 4. SC Electrical Stimulation (ES).Cyan– Strabismus angle (LE-RE); Brown – e-stim.

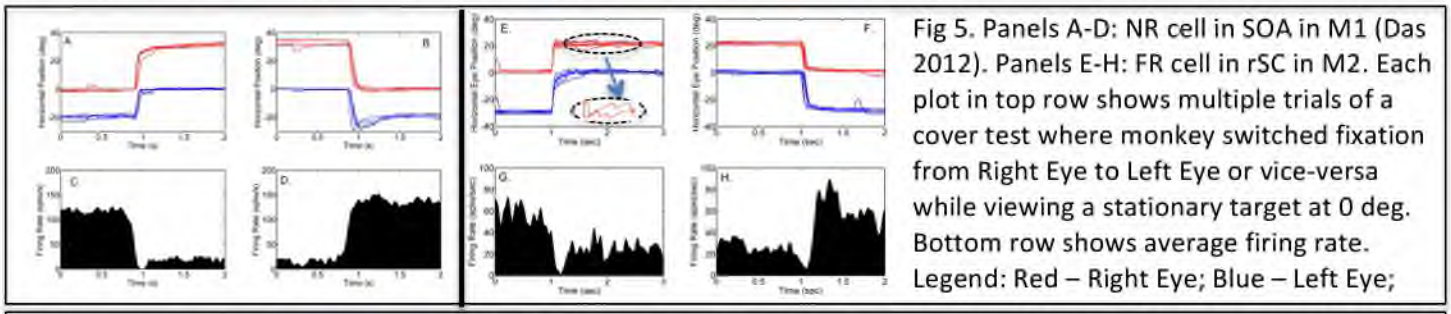

Fig 5. Panels A-D: NR cell in SOA in M1 (Das 2012). Panels E-H: FR cell in rSC in M2. Each plot in top row shows multiple trials of a cover test where monkey switched fixation from Right Eye to Left Eye or vice-versa while viewing a stationary target at 0 deg. Bottom row shows average firing rate. Legend: Red – Right Eye; Blue – Left Eye;

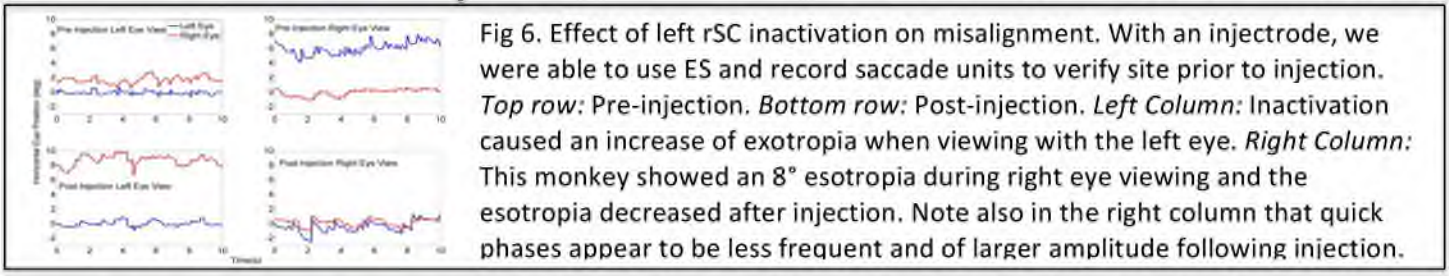

Fig 6. Effect of left rSC inactivation on misalignment. With an injectrode, we were able to use ES and record saccade units to verify site prior to injection. *Top row:* Pre-injection. *Bottom row:* Post-injection. *Left Column:* Inactivation caused an increase of exotropia when viewing with the left eye. *Right Column:* This monkey showed an 8° esotropia during right eye viewing and the esotropia decreased after injection. Note also in the right column that quick phases appear to be less frequent and of larger amplitude following injection.

Research Design

Strabismus is induced in infant monkeys using an optical prism-rearing paradigm. In this paradigm, infant monkeys view through a 20D horizontal Fresnel prism placed in front of one eye and a 20D vertical Fresnel prism in front of the fellow eye (de-correlated visual input-Fig 2). Following special rearing for 4months, the animals grow with unrestricted vision until they are 3-4 years old, at which time they are implanted with a head stabilization post, recording chamber and binocular search coils and undergo oculomotor training. Since there is a long time interval between special rearing and oculomotor experiments, it is essential that we consistently produce strabismic animals. Fortunately, the optical prism-rearing paradigm is well validated and

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our institution has excellent infrastructure to support our program to rear animals with strabismus. We will need 2-3 monkeys for each of the specific aims. Monkeys will be shared between the first two aims but not the third.

Experimental Paradigms and Data Analysis

A) We will record from rSC neurons that show activity related to strabismus angle during the following tasks

- Static fixation of targets at 3 distances (28.5cm, 57cm and 114cm) during right eye view/left eye view/binocular view (inducing change in misalignment due to change in eye of fixation; Figs 3 & 5)
- Static fixation of target at 57cm during monocular viewing through ophthalmic lenses of power +1D, 0D (plano), -1D or -2D (inducing change in misalignment due to accommodative stimulus).
- Horizontal and vertical smooth-pursuit (0.3Hz, ±15°) during monocular viewing (to induce change in misalignment due to A/V patterns and to verify that neuron does not show eye position sensitivity). Smooth-pursuit along the same trajectory as that evoked by ES will verify that the misalignment change following ES (Fig 4) is not simply due to a change in gaze position.
- A 500ms target blink condition will be introduced during fixation and during the alternating cover testing (eg: Fig 5) to examine visual response characteristics of the 'misalignment' cells. Blink testing will determine whether firing rate differences that depend on eye of fixation (Fig 5E-H) are related to visual response rather than strabismus angle. (additional details about target blink testing in Aim 2)
- Data analysis will correlate strabismus angle with firing rate under the different conditions listed above. We will also compare the rSC data with the published SOA data.

B) We will inactivate the rSC using muscimol, and examine its effect on eye misalignment and A/V patterns. Initially unilateral injections are planned. If we observe any differences between inactivation of the right rSC and the left rSC within the same monkey, then bilateral inactivation experiments will be planned. Our setup allows the simultaneous insertion of two injection pipettes. At least 2-3 injection experiments on each side of the brain will be performed.

Neural Recording & Muscimol Inactivation in Superior Colliculus

There is a wealth of knowledge about SC neural responses [66, 67]. We have conducted preliminary recordings in the SC (Figs 4, 5, 9, 14, 15) and are confident of completing these experiments. E-stim (400Hz, 10-30μamps) along with neural recording will be used to map the SC (upward saccades are medial, downward - lateral; large saccades - caudal and small saccades - rostral). Visual activity is observed in the superficial layers, immediately upon entering the SC, while saccade related activity is in intermediate and deeper layers.

Injecting muscimol leads to temporary inactivation of neurons in a local area within the brain. We will first map the target structures (rostral and caudal SC) via single unit and ES prior to inactivation experiments. A technical innovation planned is to use an injectrode, i.e., combination electrode and injection-pipette[68] (purchased from Alpha Omega Engineering). The advantage of an injectrode is that injection sites can be localized immediately prior to muscimol injection (Fig 6). 0.5-1μl of muscimol (conc. 2μg/μl) will be delivered over 2 minutes, by pressure, with a pico-pump (W.P.I. PV 830).

Expected Results & Potential Issues

We expect to identify a population of cells in the rSC that shows activity related to the strabismus angle but do not show modulation to eye position *per se*. Note that a lack of eye position sensitivity will render these cells to be not directly compatible with a Helmholtz-only framework. We predict that the rSC 'strabismus' population will show altered sensitivity and thresholds compared to the normal population (normal population sensitivity derived from Van Horn et al. 2013), thereby providing a neural basis for misalignment. Inactivating the rSC should interfere with the static bias for misalignment and therefore result in a change in strabismus angle. Fig 6 suggests a divergent change in strabismus angle, similar to what we observed following injection of the caudal fastigial nucleus (Joshi and Das 2013[11]).

We must be cautious in interpreting that the only influence of the rSC strabismus signal is on the lateral rectus (via rSC->cMRF->ABD nuc). The rSC also reciprocally projects to cerebellar areas including via brainstem pre-cerebellar nuclei (e.g., NRTP). So the rSC could also be part of a circuit that impacts the MR via the pathway shown in Fig 1. *Irrespective of the downstream circuitry, it would be a major contribution to establish that the rSC encodes a static bias signal necessary for maintaining the strabismic state. In this proposal we have restricted investigation to only the SC to maintain a cohesive plan across the three aims.*

One important question is the location of the 'misalignment' cells – Are they within the rSC? This may be an issue because the location of the rSC 'vergence' cells in normal monkeys is not histologically verified - One recent study identified vergence neurons in the rSC[31] using functional criteria while two other early

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studies[9, 29] did not explicitly localize the more dorsal and lateral (w.r.t. SOA vergence cells) vergence cells. Therefore, in addition to careful mapping of recording location and the use of the injectrode for muscimol inactivation, we will place marking electrolytic lesions at the recording sites a few days before euthanasia and perform histological reconstruction of the electrode tracks and lesion sites[11].

Aim 2: To investigate the role of the rSC in driving fixation instability

Patients with strabismus and amblyopia suffer from increased drifts and nystagmus during fixation leading to reduced fixation stability[69]. The rSC plays a role in fixation via its control over microsaccades. Therefore our aim is to identify the role of the rSC in fixation instability in strabismic monkeys.

Significance, Justification and Feasibility

Fixational Eye Movements in normals and in disease

The eyes are not still during fixation of a stationary target[3]. Tremor, drifts, microsaccades, and a slow oscillatory movement are components of fixational movements[70-72]. While fixational eye movements are necessary to prevent visual adaptation to stationary targets (e.g.: - Troxler fading effect), excessively large fixational eye movements (fixation instability) can take the fovea away from the target. In normals, fixation stability is governed by the two largest components of fixational movements (drifts and microsaccades) and both components may be critical in ensuring that the eyes maintain an optimal orientation[73, 74]. Among other factors, size, shape and luminance of the target affects the amplitude of both drifts and microsaccades[58, 75-77].

Fixation instability has been widely reported in visual disease such as AMD[78] and can interfere with day-to-day activities like reading[79]. There appears to be an inverse correlation between fixation instability and visual acuity although causality is not yet proven[80]. Fixation instability in patient populations has usually been quantified using the Bivariate Contour Ellipse Area (BCEA), which is a metric that quantifies horizontal and vertical dispersion of eye movements during fixation[81]. Although useful as a quantitative measure, the BCEA metric does not separate contributions of drift vs. microsaccades or conjugate vs. disconjugate movements towards fixation instability. A few studies in strabismic and amblyopic patient populations have also found increased fixation instability (larger BCEA)[69, 80, 82]. Perhaps a change in microsaccade rate or amplitude is present, although this may be confounded by the presence of nystagmus and the instrumentation used[69].

Role of the rSC in Fixation

The rostral pole of the SC was previously termed the 'fixation zone' based on the finding that neurons were tonically active during fixation and paused during saccades[62, 83, 84]. However recent studies showed that these neurons burst for contraversive microsaccades[63]. Therefore SC organization is a continuum from large saccades in the caudal SC to small and micro-saccades in the rostral zone[85]. A recently proposed framework is that balanced activity across populations of cells in the two rSC maintains fixation and a shift in locus of activity towards any one side will result in a microsaccade, thereby re-orienting the eyes towards the optimal location[86, 87]. Supporting this framework, pharmacological lesion of the rSC results in a reduced microsaccade rate because the number of active neurons in the rSC is reduced leading to reduced potential for imbalance[63, 86-88]. Also, an eye position offset is observed after inactivation that may be a result of attempting to reestablish the balance of activity across the two rSC necessary for optimal fixation[86]. It is not clear what would *cause* a spontaneous shift in locus of activity from one rSC to the other during fixation, but it stands to reason that the drift component of movement could be a driver, especially in pathology such as nystagmus. Other studies have suggested a link between covert attentional shifts and the generation of directed microsaccades[88-90].

Rationale for the current studies

rSC neuronal activity is correlated to quick phases of physiological nystagmus such as vestibular or optokinetic nystagmus[91] but responses during pathological nystagmus such as in strabismus or amblyopia is unknown. Our animal model presents a unique opportunity to study neural responses in oculomotor structures that might contribute towards fixation instability. Fundamentally, we are testing the hypothesis that the rSC governs the production of quick phases of nystagmus necessary to reset the eyes to optimal orientation following drift (slow phase). No nystagmus has been observed after SC lesions in normal monkeys[83, 84] suggesting that the SC is not the source of the underlying slow phases of nystagmus in strabismus. *Our strategy here will be to 1) Behaviorally characterize fixation instability in strabismic monkeys 2) Study responses of rSC neurons during fixation and identify correlations to components of fixational eye movements contributing to fixation instability 3)Study the effect of rSC muscimol inactivation on fixation stability/nystagmus.*

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Preliminary Studies

Fig 7 shows fixational eye movements in a normal and strabismic monkey. The normal animal shows stable fixation with microsaccades on the order of about 0.5°. Drifts are minimal. The strabismic monkey shows significant instability due to increased drifts and nystagmus (note amplitude scale difference in right/left column in Fig 7). Like in humans, strabismic monkey nystagmus amplitude, direction, frequency and velocity can vary from animal to animal and with viewing condition (i.e., right/left eye viewing). *We will characterize fixation instability in terms of individual fixational movements (drifts and microsaccades – nystagmus slow and quick phases) and also in terms of conjugate and disconjugate components (versional and vergence instability).*

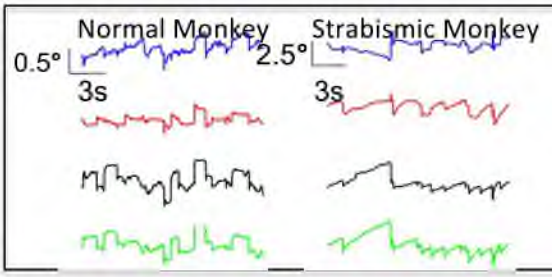

Fig 7. Fixational Eye movements in normal (left column) and strabismic monkey (right column) while viewing a 1° disk target for 15secs. Amplitude scales are different in two columns (Normal: 0.5°; Strabismic: 2.5°). Legend: Blue- Left Eye horizontal; Red – Right Eye Horizontal; Black – Left Eye Vertical; Green – Right Eye Vertical.

Fig 8 shows dispersion of horizontal and vertical eye positions during fixation in a normal (Fig 8A) and strabismic monkey (Fig 8B,C). The ellipse encompasses 68% of fixation data points and the area of this ellipse quantifies overall fixation stability (BCEA). *A larger value of BCEA indicates unstable fixation.* Ellipse parameters such as lengths and axes orientation will be quantified to understand the components contributing to fixation instability.

Since amplitudes of quick phases tend be <2°, we will record from the rSC. *Fig 9 shows rSC cell response correlated with nystagmus quick phase.* Eye traces (top four rows) shows the mean of 20 quick phases aligned on quick phase onset (vertical dashed lines). The firing rate (bottom row) shows a small burst followed by pause associated with the quick phase. There is no modulation during the slow phase (mean activity prior to quick phase is tonic). *Also tonic activity is larger for smaller exotropia (Right Eye View exotropia: ~12°; Left Eye View exotropia: ~34°), suggesting encoding of misalignment (Aim 1).*

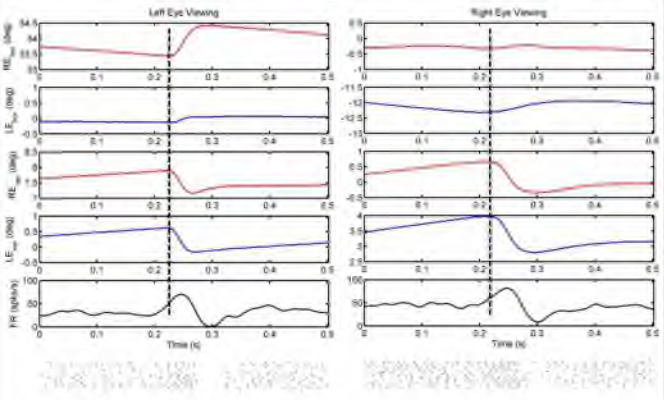

Fig 9: rSC cell response related to quick phases and to eye misalignment. Note the burst in firing that is in sync with the nystagmus quick phase and also the tonic rate that changes with fixating eye (misalignment encoding similar to Fig 5E-H).

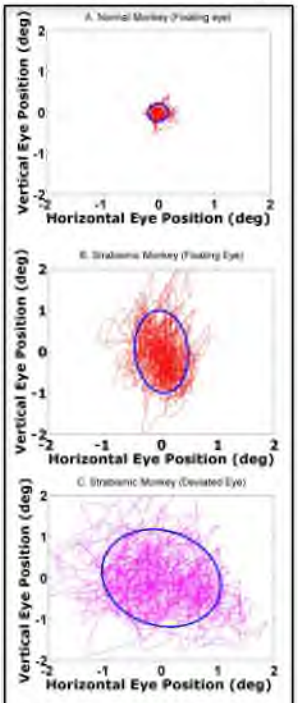

Fig 8. Fixation stability in normal and strabismic monkey (different animal from Fig 7).

A potential concern is whether the presumed rSC 'nystagmus' activity is actually a visual response to eye entering/exiting the cell's visual receptive field. The low latency between peak burst and quick phase in Fig 9 suggests that the burst is not a visual response. Additionally, we will also perform a control experiment where a brief (0.5s) target blink is introduced during fixation (Fig 10) to assess effect of vision on nystagmus and on rSC responses believed to be related to quick phases (e.g. during 2nd blink epoch in Fig 10).

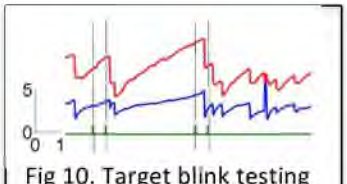

Fig 10. Target blink testing (0.5s) during fixation (10s of data). Red-RE_{hor}; Blue-LE_{hor}; Vert. line pairs- blink onset/offset.

Research Design: Experimental Paradigms

1) Binocular data will be collected as monkeys fixate a straight-ahead target (a disk target or optotype; Sizes-0.5°, 1.0°, 2.0°) during both monocular and binocular viewing as fixation stability and nystagmus can vary with viewing condition. Note that differences in nystagmus due to right eye or left eye viewing will aid in analyzing ocular specificity of rSC cells.

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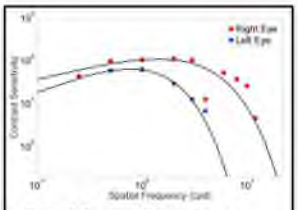
Fig 11. mCSFs in a strabismic monkey.

2) We will estimate monocular contrast sensitivity functions (mCSF – Prel. data in Fig 11) because fixation stability may be correlated with amblyopia. Animals are first trained to discriminate between two orientations[92]. Each training trial begins with central fixation followed by a 1s presentation of a central 10° Gabor pattern (1 cyc/deg; 100% contrast) oriented at ± 45°. In the response phase, the animal makes a saccade in <300ms to a right or left target depending on grating orientation (eg:- right saccade for +45° and left saccade for -45°). Once animals are trained (>95% correct for training trials), grating contrast and spatial frequency is varied in a one-down two-up interleaved staircase to identify CS thresholds for each spatial frequency[93-95]. 20-30 staircases are averaged to obtain contrast sensitivity (1/threshold contrast) at each spatial frequency.

3) Target blink testing[63], will investigate the influence of vision: - a) on fixation stability components (slow and quick phases) both before and after inactivation and b) on rSC cell responses.

Data Analysis

1) We will quantify overall fixation stability using the Bivariate Contour Ellipse Area (BCEA) metric; $BCEA = 2.291*pi*\sigma_x*\sigma_y*\sqrt{(1-p^2)}$[81]; σ_x = Standard deviation (SD) of horizontal eye position; σ_y = SD of vertical eye position, 'p' is the Pearson product moment correlation coefficient of horizontal and vertical position and 2.291 is a factor that yields an ellipse with 68% of data. We will quantify microsaccades and quick phases of nystagmus in terms of rate (microsaccades/sec), vector amplitude and direction.

2) rSC neurons show a burst of activity during microsaccades[63]. We will determine whether rSC neuron responses are correlated with the quick phases of nystagmus (Fig 9). Peri-quick phase neuronal activity will be quantified to determine if it is correlated with the drift (slow phase of nystagmus). Quick phases can sometimes be slightly disconjugate (see Fig 7&9), which will assist in analyzing ocular specificity of the cells.

3) Neuronal responses to quick phases that appear in the blink interval (no visual input; 2nd blink epoch in Fig 10) will be compared to quick phases outside the blink epoch (eg:- as in Fig 9) to assess visual response.

4) Muscimol experiments complement neural recordings in determining rSC role in fixation stability. Fixation stability (BCEA) and characteristics of fixational eye movements (nystagmus pattern, incidence of microsaccades/quick phases, velocity of slow-phases) will be compared before and after injection of muscimol.

5) SC inactivation by muscimol causes an eye position offset that potentially alters visual input from one or both eyes and therefore might influence fixation stability. To eliminate this potential influence of vision, BCEA during target blink periods (no visual input) will be compared between pre- and post-inactivation conditions.

Overlap between Aims 1 and 2

We included both aims 1 and 2 because the neuronal types implicated in each aim are in the rSC and may overlap (Fig 5, 9). It is efficient to have testing for both aims in place for when a rSC cell is isolated. Note that if an animal does not show the property of DHD (critical for misalignment studies of Aim 1), they can still be used for the fixation stability studies of Aim 2 and if animals show minimal nystagmus, they can still be used for Aim 1. We foresee no difficulty in obtaining sufficient animals for testing these aims.

One question is whether presence of nystagmus (a continuously varying eye position signal) will interfere with identification of a neuronal misalignment signal (Aim 1). The inset of Fig 5E and also Fig 9 show that nystagmus can be present and a neuronal misalignment signal can still be identified. Fundamentally, presence of nystagmus does not interfere with identification of a misalignment signal because the nystagmus is small amplitude (~1-2°) while the change in misalignment due to change of fixating eye is large (~10-15°). Also the misalignment signal is a tonic signal while the firing related to quick phases is phasic (brief burst as in Fig 9). The converse question is if presence of neuronal misalignment firing will interfere with identification of nystagmus related firing (Aim 2). Thus if the horizontal component of nystagmus is disconjugate, there could be a change in misalignment during the nystagmus and therefore neural responses due to change in misalignment could be misinterpreted as being related to nystagmus. This is unlikely to be an issue since the disconjugacy during nystagmus is usually small (<1° in Fig 9) and therefore the equivalent neural contribution (due to misalignment change) will be too small to create difficulty in interpretation. We also plan to handle this potential confound analytically as we will account for the sensitivity for eye disconjugacy (misalignment) in the neuronal response while calculating sensitivity to nystagmus slow or quick phases.

Expected Results and Potential Issues

Preliminary data shows that fixation stability in strabismic monkeys is worse than in normals. We expect to find a correlation to the level of amblyopia (estimated from the mCSF). Neural recording in the rSC is

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expected to show correlated activity with the quick phases of nystagmus (preliminary data – Fig 9). Inactivation of the rSC should decrease the incidence of quick phases (Fig 6), which could result in longer duration slow phases and possibly larger fixation instability (higher BCEA). Analysis of peri quick-phase neural activity during fixation will determine if rSC is involved in slow-phase generation. Data in Fig 9 suggests that it is not and therefore we would expect slow phase velocity to be unchanged following muscimol inactivation.

It could be argued that the fixation framework is inappropriate because quick phases are typically larger than microsaccades. We have used this framework because the nystagmus is observed during fixation and the quick phases could be reorienting the eyes to an optimal location. We will analyze eye orientations at the end of the quick phase and as we sample the rSC, we will be able to identify and compare rSC cells responses to true microsaccades (<1°) with the rSC cells responses to quick phases.

Spread of muscimol to the adjacent Nucleus of the Optic Tract (NOT) could induce a nystagmus with contralateral slow phases. However this is not a major concern as many studies have inactivated the SC including the very rostral pole without inducing nystagmus[84, 86, 87, 96-103]. The strategy is to inject small volumes (1-2µl) and to consider lesion effects in the first hour after injection (eg:- ref 84). We will use the same strategy further aided by the use of an *injectrode* that improves localization and allows smaller injection volumes. Note that nystagmus due to NOT lesion is purely horizontal and so can be distinguished from any post rSC lesion induced changes in the multi-planar nystagmus (Fig 7) that is usually present in the strabismic monkeys. Preliminary data in Fig 6 (esp. left column) shows that we can inactivate the rSC without inactivating the NOT.

Aim 3: To investigate the role of the SC in fixation-switch behavior

Many strabismics can change their eye of fixation depending on target location. Our goal is to study the role of visual, visuomotor and motor cells in the SC in driving fixation-switch in strabismic monkeys.

Significance, Justification and Feasibility

Strabismic patients with unilateral amblyopia will fixate and acquire targets with the normal or non-amblyopic eye. However if amblyopia is minimal, patients with strabismus develop the ability to fixate targets with either eye and can spontaneously change the eye of fixation depending on the location of the target[104-107]. A saccadic eye movement that results in fixation-switch is called an alternating saccade[104, 105, 108, 109]. Fixation-switch is likely driven by visual suppression of specific retinal areas of each eye although mechanisms are yet undetermined[104-106]. Behavioral studies in human exotropes and metabolic studies in monkeys with exotropia suggest that portions of the temporal hemi-retinae are suppressed[42, 105, 110]. A recent study showed that the fovea of the deviated eye and parts of the temporal retina adjacent to the fovea was not suppressed[111]. Esotropes who show alternating cross-fixation might demonstrate suppression of the nasal hemi-retinae of the two eyes[105, 112]. *Strabismic monkeys, whose strabismus was induced by either surgical[44] or sensory methods[108, 113] show fixation-switch behavior making them a good model system to understand this unusual visual-oculomotor behavior. We have shown that spatial fixation patterns and fixation-switch behavior followed expectations if portions of the temporal (nasal) retina were suppressed in exotropia (esotropia)[108, 113].*

Possible frameworks for understanding fixation-switch behavior

A *target-selection framework* could possibly be used to explain fixation-switch behavior. In target selection in normals, the 'cyclopean' brain has access to retinal error signals from multiple targets and must make a decision of which target has highest priority or salience and make a saccade to it. The FEF, LIP and SC are critical parts of the target selection circuit[114-116]. *In strabismus*, the eyes are pointing in different directions and so a single target will theoretically produce two retinal error signals. Although the subject perceives only one target (due to visual suppression), our hypothesis within this framework would be that the oculomotor system has access to both errors[60, 105, 113]. Spatial fixation patterns in strabismus can then be explained within a 'target-selection' decision framework wherein the brain chooses between the two errors to prepare a saccade. For eccentric target locations the strength of the retinal error representation from one of the eyes is significantly stronger (fellow-eye suppression is strong) leading to an 'easy' decision of selecting which eye to use to acquire the target (Fig 12A,B). For target locations approximately midway between the two foveae, strength of retinal error representations from each eye is similar leading to a 'hard' choice and therefore trial-to-trial variability in choice of fixating eye (Fig 12C). Analysis of saccade reaction time supported this hypothesis as the 'harder' choice led to longer latency[113]. An alternative framework would involve bottom-up processing resulting in only one retinal error available to the oculomotor system. *We propose to test the validity of these frameworks for fixation-switch by examining neural responses in the caudal SC.*

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Evidence that the SC plays a role in target selection

A substantial body of work has shown that the SC is a critical part of the target selection circuitry[66] and only a minimal review is provided here. Build-up cells in the SC are most involved in target selection[117-119]. These cells show visual activity whenever a potential target enters its receptive field. Following visual activity, there is a build-up of background firing prior to a second burst of activity that is correlated with the saccadic eye movement[120]. Target selection occurs during the build-up period, as evidenced by the observation that build-up activity was greater when a saccade was made to the target in the receptive field (i.e., visual target and selected target is same) compared to a saccade made to a target outside the receptive field (i.e., visual target and selected target are different)[118, 121, 122]. Focal inactivation of the SC using muscimol can bias target selection away from the inactivated area[100] while sub-threshold electrical stimulation can bias selection of targets towards the stimulated site[123]. *The known pattern of SC cell responses during target selection in normals can be used to test whether this framework applies when selecting eye to fixate a target in strabismus.*

Preliminary Studies

Fig 12 shows an example of fixation switch in an *esotropic* monkey during binocular viewing. Target steps into the temporal retina of the previously fixating eye never results in a fixation switch (Fig 12A; left eye fixating) and target steps into the temporal retina of the previously non-fixating eye results in a 100% fixation switch (Fig 12B; left eye fixation to right eye fixation). Target steps that place the target in between the gaze axes of the eyes (Fig 12C) are interesting as the animal switches fixation on some trials (left eye to right eye in Fig 12C) but maintain the previously fixating eye (left eye) on other trials.

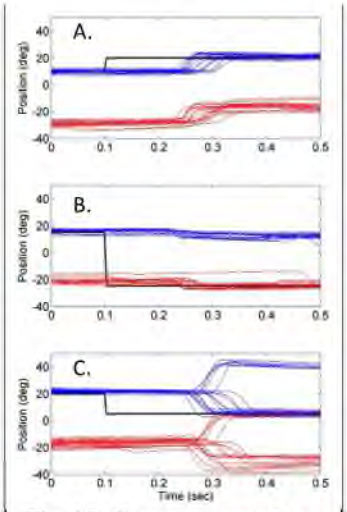

Fig 12: Eye movements during binocular viewing saccade task. Red – Right Eye; Blue: Left Eye; Black – Target.

Fig 13 summarizes data from over 2000 saccades in an esotropic monkey showing spatial fixation patterns (i.e., which eye was used to acquire the target) based on the retinotopic location of the target (w.r.t. fixating eye at time of target step). Warmer colors indicate greater incidence of fixation with the right eye. Fixation-switch occurs for horizontal target locations that are approximately greater than halfway between the lines of sight of the foveating and strabismic eyes (location where color changes from red to blue). The border between right eye and left eye fixation zones is not sharply defined and there is a significant extent (>10°) over which the monkeys could acquire a target with either eye (gradual shift from warmer to cooler colors; Fig 12C shows one instance).

Our plan is to examine SC activity during saccades like those in Figs 12,13 and determine what drives the decision of choosing either the right or left eye to saccade onto the target. Fig 14 shows activity of a saccade burst neuron with an upward preferred direction (30° exotropia, binocular viewing, right eye fixating at target onset). The left column shows 'no fixation-switch' trials in which the target moved upward (5°) and the right eye was fixating. The right column shows trials in which the target stepped upward (5°) and also to the left (30°). Here the animal made a 5° up saccade but in addition switched fixation from right to left eye. In both cases, the burst neuron encodes the vertical saccade. The rather low firing rates may be specific to strabismus or a reflection of a previous finding that SC firing rates are depressed when multiple targets are presented[117]; although this effect was only reported in buildup cells.

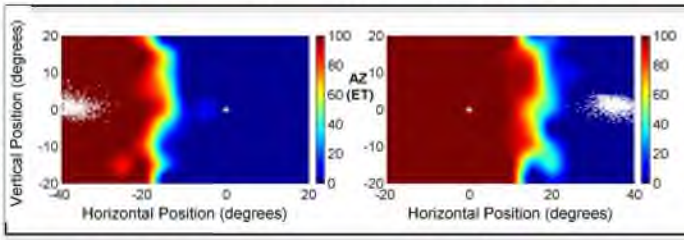

Fig 13: Spatial fixation pattern based on retinotopic presentation of target (Agaoglu et al., 2014b). Left Panel: Trials in which left eye was fixating at time of target step); Right panel: Right eye fixating trials. Legend: '+' - Position of the previously fixating eye; 'dots' - Position of the previously non-fixating eye.

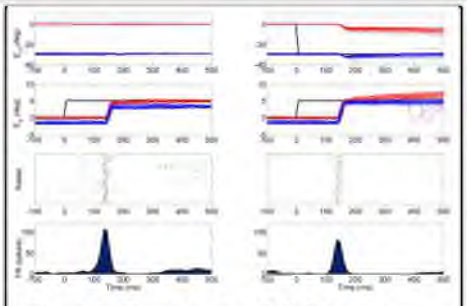

Fig 14. Example of SC burst neuron. Red-right eye; Blue-left eye; Eh: Horizontal position; Ev: Vertical position; Black – Target

SC visuo-motor cells are important for target selection *and could also be critical in determining which eye is used to acquire the target in strabismus.* Fig 15 shows a build-up cell while an exotropic monkey performed a delayed saccade task to a target presented at a down and right 5°

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location (monocular RE viewing; 0.5s overlap; only RE hor and ver position is shown). The cell shows visual activity ~60ms after the target appears in the visual receptive field (left column). There is also a burst of activity associated with the saccade to the target (right column). In the interval between the visual and saccade related activity, there is increased firing (build-up activity; right col.) that could be related to decision-making. *We will test the hypothesis that build-up activity is different for trials involving fixation-switch where visual/saccade vectors can differ Vs trials that do not involve fixation switch and visual/saccade vectors are same.*

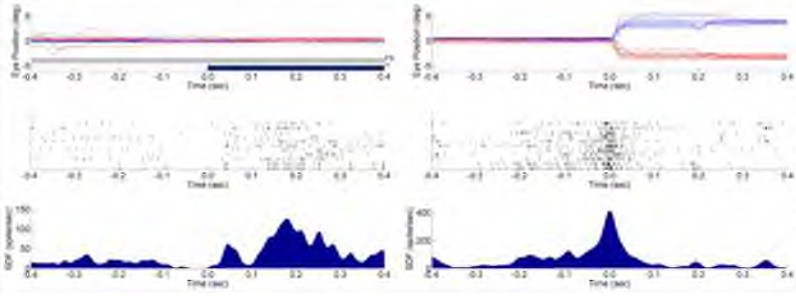
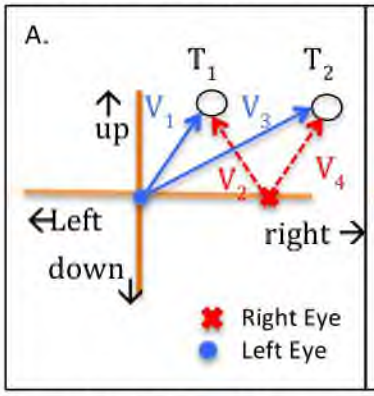


Fig 15. SC build-up cell activity in a delayed saccade task. Left column: trials aligned on target onset. Neural activity is visual response in overlap period due to presence of target in visual receptive field (before saccade onset). Right column: aligned on saccade onset. Build-up of activity begins ~200ms before the saccade followed by a saccade-related burst. *Legend: Blue- Right (viewing) eye horizontal position; Red- Right eye vertical Position; FS-Fixation Spot; T- target.*

Research Design - Experimental Paradigms and Data Analysis

The following expts will characterize SC cell activity during saccade tasks that may involve fixation-switch.

1) *Mapping and Tuning Curves (Monocular viewing):* A 'Delayed Saccade Paradigm'[120] is first used as a search stimulus to identify visual receptive field location and optimal saccade vector for an isolated cell. Vectorial target amplitudes from 5-25° (5° steps) and contralateral directions (separated by 15°) will be tested. In practice, only a subset of directions need be tested since previous ES mapping helps to narrow to one quadrant. Mapping is critical since SC cells fire briskly only for saccades to optimal target location. A typical trial begins with the monkey viewing a fixation spot ('FS'). After a random time interval a second target spot ('T') appears in the periphery. The monkey maintains fixation on the FS until it disappears at which time the monkey initiates a saccade to the target spot ('T'). This paradigm is preferred because it introduces an overlap time interval (0.5s-1s) that separates the visual, build-up and saccade related responses of the cell (Fig 15), simplifying analysis. *Tuning curves for each monocular viewing condition will be constructed to identify ocular specificity within SC cells (found in other structures such as the PPRF*[12, 124]*). Any disconjugacy in the movement of the two eyes will help in identifying ocular specificity.*
2) *Binocular Testing:* Once the optimal vector is identified, the delayed saccade paradigm during binocular viewing will commence to determine neural processing leading to 'fixation-switch' or 'no fixation-switch' behavior. Fig 16A shows a schematic of a binocular viewing experiment in which the animal is initially viewing the FS with his left eye (right eye deviated to simulate exotropia). We will randomly present a target at T_1 (receptive field location estimated during monocular viewing) or T_2 (corresponding putative receptive field location with respect to deviated right eye). Also shown in Fig 16A are the possible eye movement vectors (V_{1-4}) that would bring either the left eye (V_1 and V_3 – no fixation switch) or the right eye (V_2 and V_4 - fixation switch) onto the target. It is possible that during binocular viewing, the animal prefers to initially fixate the FS with his right eye (left eye deviated to left). In this case, the schematic of possible target locations and eye movement vectors is as in Fig 16B. Here, the fixation-switch vectors would be V_1 and V_3; V_2 and V_4 would be 'no fixation-switch'. A specific strategy to ensure an initial condition of either right eye or left eye fixating would be to horizontally offset the initial FS position to the right or the left.



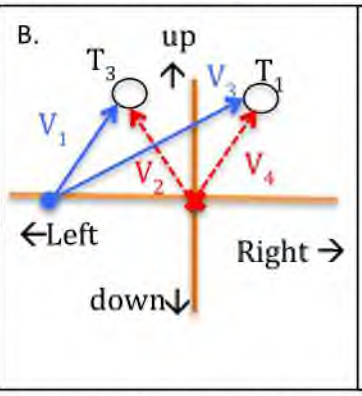


Fig 16: Simulation of target presentation locations and eye movement vectors during SC recording. Panel A-Monkey fixating the fixation spot(FS) with the left eye (blue circle); right eye (red 'X') is deviated to the right. Target is presented at visual receptive field location either at T_1 (RF w.r.t left eye) or T_2 (putative RF w.r.t deviated right eye). Possible eye movement vectors are V_1-V_4. V_1, V_3 bring left eye (blue) to target (no fixation switch) and V_2, V_4 bring right eye (red) to target (fixation switch). Panel B- target locations (T_1, T_3 at visual RF) and movement vectors V_1-V_4 when the right eye is initially fixating the FS.

3) *Data Analysis and Expected Results:* To illustrate our data analysis process, consider the cell and initial conditions depicted in Fig 16A. Trials are sorted according to target location (T_1, T_2) and then further by eye

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movement vectors (V_1 or V_2 for T_1; V_3 or V_4 for T_2). We will calculate and compare visual, build-up and saccade burst activity in each condition (Note that the analytical methods will be basically adapted from studies on target selection- e.g., McPeek and Keller[121, 125] , Thompson et al.[126]). Visual Response – Trials will be aligned on target onset and average and peak visual response and response latency to stimulus onset calculated. *A neural visual response to both T_1 and T_2 will indicate that visual information is available via both the fixating and the deviated eye.* However the magnitude of the visual response could vary depending on whether the target is in the receptive field of the fixating eye or the deviated eye (e.g., T_1>T_2). Saccade Response – Trials will be aligned on saccade onset and peak saccade response and response latency to saccade onset calculated. Peak saccade burst response is expected to correspond to the appropriate saccade vector irrespective of whether the right or left eye acquires the target (i.e., V_1 or V_4 only in Fig 16A; also shown in Fig 14). Buildup Response – Trials will be aligned to saccade onset and buildup response analyzed as mean response over a period -200ms to -50ms prior to saccade onset (Fig 15). In addition, we will implement the elegant 'area under the ROC curve' method used by McPeek and Keller and Thompson et al. to identify the time course of decision-making leading to one or the other eye acquiring the target. We will correlate this 'decision time' parameter with both saccade latency and with the probability of 'fixation-switch' or 'no fixation-switch'. The buildup activity is expected to encode decision information – for example, when target is presented at T_1, mean buildup activity in V_1 trials may be greater than mean buildup activity in V_2 trials; For T_2, buildup activity in V_4>V_3. For certain target locations, animals may never switch fixation or always switch fixation (eg:- only V_1 trials for T_1 and only V_4 trials for T_2 are obtained in Fig 16A; Fig 12A,B). *Comparison of firing characteristics in V_1 and V_4 trials will provide insight into efficacy of information processing when visual information is processed via the deviated eye. Alternatively this comparison will determine if visual target information is obtained via only one eye (the previously fixating eye) and then transformed to yield a saccade bringing the fellow eye onto the target.*

4) *Monocular testing:* Testing using the same target locations (T_1 and T_2) will also be performed during monocular viewing. In monocular viewing trials, we would expect the viewing eye to also acquire the target (eg:- V_1 and V_3 in Fig 16A). However Economides and colleagues[127] recently reported the presence of 'crossover saccades' in human exotropes, which they defined as saccades where a fixation switch is observed even when the deviated eye did not 'see' the target (e.g., during dichoptic or monocular viewing). Vectors V_2 and V_4 are examples of crossover saccades during monocular viewing. If crossover saccades occur in the monkeys, it would be interesting to study neural characteristics that lead to these saccades.
5) *Innovation - Dual recording:* An innovation planned for this aim is to perform dual electrode recording to simultaneously record from pairs of collicular neurons. For example, in Fig 16A (T_1 target), vector V_1 would be encoded in left SC and V_2 in the right SC. Simultaneous recording from both colliculi will provide the opportunity to directly compare the temporal characteristics of neuronal responses in trials in which the monkey chose to make a leftward saccade or a rightward saccade to acquire the target.
6) *Innovation – Placement of targets in visual receptive field:* Drifts and trial-to-trial variation in deviated eye position could present a problem when presenting the target at the optimal visual receptive field with respect to the deviated eye (e.g. T_2 in Fig 16A). To overcome this issue, we will calculate the desired T_2 target location as the sum of the T_1 location (RF w.r.t to the fixating eye) and the *measured* position of the deviated eye at time of target presentation. This is similar to image stabilization except that the T_2 target location is not updated continuously (calculated once per trial). There is a one-frame (8.33ms) delay in updating the T_2 location but eye drifts in this time is too small to result in incorrect presentation of the target.
7) *Alternative Hypothesis and Other Potential Issues:* An alternative to the competitive decision framework is to consider that bottom-up processing results in only one retinal error being represented in the SC. It could be that visual signals that correspond to only a) the previously fixating eye or b) the eye that will eventually acquire the target could be represented in the SC cells. Recording SC responses can differentiate between these alternatives. For dual recording, placement of the target will be somewhat limited by the preferred saccade vector (receptive field location) of each of the rSC cells. If these vectors do not match properly (e.g., V_1, V_2), a trial-by-trial analysis of temporal characteristics may not be possible. However, dual electrode recordings will still be fruitful because they will serve to rapidly increase the number of cells associated with these experiments. Fundamentally dual recording has the potential to enhance analysis but is not critical for success.

Timetable for Project

	Yr1	Yr2	Yr3	Yr4	Yr5
Aim 1: Analysis of misalignment responses	XX	XX	XX		
Aim 2: Fixation instability and nystagmus	XX	XX	XX		
Aim 3: Fixation-switch behavior			XX	XX	XX

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Vertebrate Animals

1) Description of Animal Use

All of our studies conform to NIH guidelines for the care and use of non-human primates. All of our procedures are reviewed and approved by the University of Houston, Institutional Animal Care and Use Committee. The PI is currently a review panel member of the University of Houston IACUC and is keenly aware of animal care and use issues. We only use approved standard operating procedures (SOP) for handling non-human primates. We typically have two to four rhesus monkeys (Macaca mulatta) for chronic recording in each year of this study. We must also raise and maintain specially reared cases for use in future recording projects. Additional strabismic animals (usually 2-4) are reared in the initial years of the project to account for unforeseen loss of animals that may occur. Animals are used as subjects in chronic single unit recording studies for approximately 2-3 years, preceeded by 2-3 months associated with surgical preparation and behavioral training.

Animal Enrichment: University of Houston maintains an enrichment program for all non-human primates. All of our animals are provided with enrichment on a daily basis. Compatible animals are caged together whenever possible and always receive visual contact and at least some tactile contact with neighbors when possible. Sometimes the extent of physical contact must be limited to prevent animals from damaging surgical implants. All animals are provided with toys and foraging boards for increased enrichment.

Infant Rearing: We are able to obtain all of our infants on the first day of postnatal life. These infant animals are born at [redacted] a MD Anderson research facility at Bastrop TX. The animals are reared for the first 2 weeks at the Bastrop facility using special rearing conditions specified in the proposal - Monkeys are raised with disrupted binocular visual experience produced by prism wearing. At 2 weeks, they are transferred to the University of Houston, College of Optometry. The special rearing condition is unchanged. Infants are housed in UHCO nursery and checked at least twice a day, 7 days/week by laboratory and animal care staff to make sure that prism goggles are in place and in good condition. Infant monkeys receive eye exams during the first month of life to screen for any congenital ocular problems. General infant husbandry (feeding, incubator maintenance and cleaning) are handled by UHCO animal care technicians. These services are covered under the UH standard nursery per diem rate. UH veterinarians with extensive experience with macaque infants provide any necessary clinical support for our infants. However, we provide all of the care associated with prism goggle maintenance. Infant monkeys receive extensive handling in the first months of life.

Surgical Procedures (Juvenile macaques): The P.I. is responsible for all surgical procedures and trains all laboratory personnel in appropriate surgical technique. We provide 2-3 weeks of adaptation to the primate chair and pole handling prior to surgery. All surgical procedures follow established protocols in our laboratory. Full aseptic surgical technique is employed in a dedicated surgery facility (see Animal Use Statement below). Rhesus monkeys are prepared for surgery, which consists of three parts: 1) Eye coils for accurate measurement of eye position are implanted underneath the conjunctiva of both eyes in each animal (Judge, et al., 1980). 2) A custom made titanium head post is attached to the skull for stabilization of the head during behavioral training and recording sessions. 3) Custom made titanium chambers for single unit recording are stereotaxically implanted, on the skull, over a hole centered above the area of interest. The head post is directly screwed onto the skull using titanium screws (Adams et al. 2007) while recording chambers are attached to the skull with dental acrylic. All surgical procedures are performed under general anesthesia (Isoflurane 1.2 - 2%). Post-surgical treatment is provided during the week after surgery to reduce inflammation (e.g., Banamine or meloxicam) and provide analgesia (e.g., Buprenorphine). Prophylactic antibiotic treatment if deemed necessary is provided by the Veterinarian staff.

2) Justification for Choice of Species and Number of Animals Used

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Rhesus monkeys have been selected for use for several reasons. First, the large database describing visual and oculomotor system function already available in the research community, as well as our own experience make it an ideal animal for study. Second, the similarity between the oculomotor and visual systems of the rhesus monkey and humans make the macaque an appropriate choice for attempting investigations on motor consequences of strabismus. Finally, we have been able to develop an animal model for strabismus using the prism-rearing method in the rhesus monkey. This allows our work to provide valuable insights important for diagnosis and treatment of different types of strabismus. Monkeys will be used across the different aims whenever possible because the behavior and neural recordings are interrelated. The basic strategy is to use monkeys on multiple specific aims wherever possible to maximize efficiency. This strategy is also necessary in the spirit of humane use of animals to reduce unnecessary excessive use of animals and minimize undue stress.

3) Veterinary Care Information

The following explains University of Houston animal welfare compliance: The University of Houston has the necessary facilities for the care and maintenance of non-human primates. The University of Houston operates to comply with the USDA Animal Welfare Act (Public law 89-544) as amended by PL91-579 (1970) PL94-279 (1976) and 45 CFR37618 (6-30-80); Health Research Extension Act of 1985 (Public Law 99-158); follows the Public Health Service Policy on Humane Care and Use of Laboratory Animals (revised September 1986); and the Guide for the Care and Use of Laboratory Animals DHEW (NIH) 85-23 revised 1985. University of Houston is a registered Research Facility under the Animal Welfare Act. The University of Houston holds an Assurance Statement on file with the National Institutes of Health Office of Laboratory Animal Welfare (OLAW). The Assurance number is A3136-01. University of Houston is accredited by AALAC. University of Houston animal operations is under the direction of a Doctor of Veterinary Medicine and staffed by personnel with training and experience in laboratory animal medicine, surgery, clinical care and diagnostic pathology. The animals are kept in cages in climate controlled quarters and are inspected daily.

4) Procedures to Minimize Stress

All surgical procedures are performed under full sterile conditions in a dedicated surgery room maintained by the University of Houston Animal Operations. Animals are anesthetized with isofluorane (1.5 - 2%) for longer procedures or Ketamine for shorter procedures. All of these anesthetics allow rapid post-surgical recovery in a recovery room under observation of veterinary staff and experimenter. Post-surgical anti-inflammatory and analgesic agents (e.g., Banamine and Buprenorphine) are administered for the first few days after surgery.

Our monkeys are adapted to handling and chairing before surgery. During behavioral training and single unit recording the monkeys head is stabilized and he sits in a primate chair facing a tangent screen. The animals work for an applesauce or juice reward during training and recording. Sometimes, the animal's solid food is reduced to motivate him to work for food during the experimental session. The monkeys used in these experiments gain weight at rates comparable to age matched, non-experimental animals. All procedures are reviewed and approved by the UH IACUC before implementation.

5) Method of Euthanasia

At the end of an experimental series, animals are prepared for euthanasia as follows. Pre anesthesia is accomplished with a single injection of Ketamine (25 mg/kg I.M.). Animals are then given a lethal dose of barbiturate (Nembutal 90 mg/kg I.V., (i.e., 3 times the surgical anesthetic dose). Immediately following this lethal barbiturate dose, animals are perfused, transcardially, with saline followed by paraformaldehyde fixation and sucrose cryoprotection to allow histological processing.

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