

STANDARD PROCEDURE
for
ANIMAL CARE and USE PROCEDURES
REVIEW

All animal care and use procedures (ACUPs) involving animals covered under the regulations of the Animal Welfare Act must be approved by the Institute Animal Care and Use Committee (IACUC) **before** such procedures commence. All submissions, reviews and retention of documents relating to ACUPs will be handled in such a manner as to comply with applicable federal regulations. The standard procedures associated with the review of Animal Care and Use Procedures are given below:

I. SUBMISSIONS AND VETERINARY CONSULTATION

All animal care and use procedures at Rose-Hulman Institute of Technology are to be documented on an ACUP Form (see Attachment A). The person responsible for the submission of the written procedures is termed the Principal Investigator (PI). The PI is defined as *"..... an employee of Rose-Hulman Institute of Technology, or other person associated with Rose-Hulman, responsible for a proposal to conduct experimental research or to conduct an experiment or demonstration for teaching purposes and for design and implementation of such experiments involving animals"*. The form requests the information necessary for review by the Institute Animal Care and Use Committee (IACUC).

The law requires that an Attending Veterinarian (AV) or his/her designee be consulted by the PI when planning procedures in order to provide guidance in matters of animal medicine/technology and information necessary to complete immobilization, anesthesia, analgesia, tranquilization, euthanasia, and adequate pre-procedural and post-procedural care in accordance with current established veterinary medical and nursing procedures. The PI is to consult with the attending veterinarian prior to completing the form.

Once the form is completed, it will be recorded and forwarded to the IACUC Secretary for final review/consultation and assigned an ACUP number.

II. ACUP REVIEW by the IACUC

The procedures and associated information contained within the completed ACUP form are to be reviewed by the full IACUC or by at least two members of the IACUC acting as a subcommittee. Many ACUP reviews will be performed by subcommittee.

USDA regulations require that all members of the IACUC have the opportunity to request and receive a full IACUC review of any ACUP. Consequently, each ACUP will be provided to all IACUC members five (5) working days in advance of the Subcommittee review. Unless one of the IACUC members requests full committee review, the ACUP review will be handled by the subcommittee. If there is a request for full IACUC review, arrangements for that review will be made by the IACUC Chairman or his/her designee.

There will be an exception to the five day notice period for urgent ACUPs to be reviewed by the IACUC. In such cases, a telephone poll will be conducted to determine whether any committee member desires full IACUC review.

All ACUPs will be reviewed by either the IACUC or its subcommittee, and reviews will be conducted in the following manner:

1. The Chairman or his/her designee will specify a primary and secondary reviewer for each ACUP. All IACUC members will receive a copy of the ACUP Abstract with reviewers designated on the form.

2. Primary and secondary reviewers will receive a copy of the ACUP at least five working days in advance of any IACUC or subcommittee review; in the case of the expedited review a copy of the ACUP may be provided with less lead time. It is the reviewers' responsibility to evaluate the procedures and other information, including making direct contact with the PI for explanation of procedures unfamiliar to the reviewer or for other clarifying information.
3. The reviewers will decide the ACUP's compliance with USDA regulations and provide comments/approval to the IACUC Chairman. Such approval shall, at the discretion of the Chairman, extend only until the IACUC has an opportunity to review the ACUP. The reviewers shall return the reviewer's minutes form to the IACUC Chairman who will notify the PI of the IACUC action and record the action in the IACUC record.

The vote may result in the following:

- i) APPROVAL
- ii) WITHHOLD APPROVAL
- iii) REQUIRED MODIFICATIONS
(Returned to PI with list of modifications required for approval.)

The IACUC Chairman or his/her designee is responsible for recording the minutes of the review.

4. In the case of full IACUC review, the primary reviewer is responsible for presenting the ACUP to the IACUC. The ACUP will then be discussed and upon conclusion of the discussion, a vote of IACUC members in attendance is taken. The PI will not be present for the discussion and the vote of approval, although he/she may make themselves available to answer questions.

III. NOTIFICATION TO PRINCIPAL INVESTIGATOR

The Chairman is responsible for notifying the PI in writing of the action taken by the reviewing body as soon as reasonably possible. If the ACUP is approved, the notification will include the ACUP number that has been assigned.

IV. RESUBMISSION OF ACUPS

A. Required Modifications

ACUPs requiring modification will be returned to the PI to be amended and resubmitted into the ACUP review process. The review and notification procedure are the same as mentioned previously.

B. Annual Review of ACUPS

All ACUPs will undergo periodic review, at least annually, after their initial submission. The IACUC will establish the review cycle for each ACUP and at its discretion may require more frequent review of some ACUPs. In either case the

PI will be notified of the review requirement. He/she will then be responsible for updating the ACUP's information and submitting for review as outlined above.

C. Substantive Changes in ACUP

The ACUP is specific and unique in a number of ways including animal species, persons performing procedures, techniques to be used, method of euthanasia (if required), method of anesthesia (if required), etc. If there are changes in these or any other substantive information within the ACUP, the PI is required to modify the ACUP and submit it as a new procedure.

V. IACUC CONSIDERATION

A majority of the IACUC will hold periodic meetings to consider the actions of the ACUP Subcommittee. All members at this session of the IACUC will have an additional opportunity to ask questions concerning individual ACUPs, and the Subcommittee will discuss significant issues that may have arisen during the ACUP review process. At the close of discussions the IACUC will affirm or disaffirm the actions of the ACUP Subcommittee. If the IACUC decisions affect individual ACUPs, the Chairman of the IACUC will notify the appropriate PI of the need to update or amend the ACUP in question.

List of Attachments

Attachment A: Animal Care and Use Procedure (ACUP) Form

Attachment B: Review and Evaluation Form

Attachment C: Notification Form

ATTACHMENT A

ANIMAL CARE AND USE PROCEDURES (ACUP) FORM

Purpose of Proposed Research or Class Experiment/Demonstration

Briefly explain in language understandable to a layperson the aim of the research or teaching experiment/demonstration proposed, including why the research or teaching experiment/demonstration is important to human or animal health, the advancement of knowledge, or the good of society.

This research program's major goal is to increase understanding of the control system that links retinal metabolic load with nutrient supply from the retinal circulation. In the experiments proposed here, two different methods of measurement of retinal blood flow, the fluorescent microsphere method and the particle tracking method, will be used to make blood flow measurements in rats during normal conditions and during photic stimulation of the retina. The specific aims of this study are 1) Modification and characterization of the particle tracking method for measurement of blood flow through the retinal circulation of the rat, 2) Modification and characterization of the fluorescent microsphere method for the measurement of blood flow through the retinal circulation of the rat, 3) Characterization of the effect of flicker stimulation of the retina on retinal blood flow, and 4) Determination of the role that action potential generation in the inner retina plays in triggering increased blood flow during retinal stimulation.

The microsphere method for blood flow measurement is a technique in which labeled microspheres are injected into the left ventricle of an anesthetized rat and become embedded in the retina. After the animals are sacrificed, the retina is removed and wholemounted, and the spheres are counted using a fluorescent microscope. In the particle tracking technique, very small microspheres (roughly 2 μm in diameter) are injected intravenously. These particles are imaged using a microscope with a fluorescence attachment. Images are recorded on sVHS videotape and blood velocities and flows are calculated.

Once the variability and reproducibility of these measurement techniques are optimized for use in the rat retina, the effects of neural activity on retinal blood flow will be investigated. First of all, the increase in retinal blood flow that accompanies flicker stimulation will be characterized in the rat retina. In later experiments, the Na^+/K^+ pump blocker TTX will be used to block action potential generation in inner retinal neurons to see if this activity triggers the increase in retinal blood flow that occurs in response to retinal stimulation.

Rationale for Animal Use

- 1) Explain your rationale for animal use, including reasons why non-animal models cannot be used.
- 2) Justify the appropriateness of the species selected. [Nb. The species selected should be the lowest possible on the phylogenetic scale.]

1) The control of blood flow relies on the interplay of several different chemical messengers, some of which may still be unidentified, arising from multiple cell types in the retina. This type of interrelationship cannot be duplicated in non-animal models.

2) Rats have a vascular retina that is similar in many respects to the human retina. Mouse eyes would be significantly smaller, multiplying the technical difficulties in the recordings. It would be difficult to assess whether results obtained from non-mammalian species would be applicable to humans.

Experimental Protocol/Time Line

Provide a description of all procedures using animals. For animals undergoing non-survival surgery solely for tissue procurement, a short description of the procedures for obtaining tissue and a brief description of the usage planned for the tissues will be adequate. A description of restraint and anesthesia protocols must be provided in sections IV and V, respectively, and non-surgical and surgical procedures must be provided in sections VI and VII, respectively. These details need not be provided here.

Provide a breakdown of the experimental groups and an experimental protocol for all procedures using animals. For animals undergoing survival surgery, multiple manipulations, and/or repeated observation, a time line is necessary. The time line should begin with animal procurement, denote the approximate timing of all important manipulations, and conclude with final disposition.

Adult Long-Evans (*Rattus norvegicus*) rats will be used for these studies. Approximately 60 animals of either gender, each weighing around 300g, will be used for all 4 specific aims of the grant proposal **which covers a period of three years**. Therefore it is expected that these experiments will use a total of 20 rats per year. There is, however, a possibility that more animals will be needed for the last year of the grant period. This is dependent on the results of the first two specific aims in which the measurement techniques, which include the microsphere technique and the particle tracking technique, will be characterized. Depending on the inherent variability of these measurement techniques, more animals may be requested for the last year of the proposal.

Animals will be housed two to a cage in the animal care facility in Olin Hall on the Rose-Hulman campus. Veterinary care will be provided by the Institute Veterinarian, James Holscher D.V.M. Animals will be deprived of food for 12 hours before experiments.

General Surgical Procedures

Animals will be anesthetized by one of two methods. The first is subcutaneous injection of urethane anesthetic (1.6 g/kg), which is a slow, but extremely long-lasting anesthetic. Animals anesthetized with this technique will take roughly 1-2 hours to reach surgical levels of anesthesia. An alternative form of induction anesthetic will be 100 mg/kg of a 50-50% mixture of ketamine and xylazine injected intraperitoneally, followed by infusion of urethane anesthetic (200 mg/kg loading dose, 100-200 mg/hr maintenance dose). This will result in much faster induction of anesthesia. During surgery, the depth of anesthesia will be monitored using cardiovascular parameters as well as by using the pinch test on a forelimb. As the initial anesthetic wears off, sodium pentobarbital (5%,

dosage sufficient to provide anesthesia) will be injected to maintain surgical depth of anesthesia. During surgery, the animal will be placed on a heating blanket and body temperature will be kept at normal levels. Also during surgery, the ECG and oxygen saturation will be monitored continually.

After induction of anesthesia, cannulae will be placed in the femoral artery and vein of the rat. The arterial cannula will be connected to a strain gage transducer to allow monitoring of systemic blood pressure. A tracheostomy will be performed and the animal will be intubated to facilitate later ventilation of the animal. Xylocaine gel anesthetic will be placed in the ear canals of the animal. Once prepared, the animal will be placed in a modified stereotaxic instrument, and ear bars will be inserted into the anesthetized ear canals for stabilization of the head.

If vitreal injections of drugs are required, surgery will be done to expose the eye and if necessary the eye will be placed on an eyering using suture. If no vitreal injections are required the eye will be wetted with artificial tears and closed. During retinal stimulation, eyelids will be retracted using a speculum. Whether or not eye surgery is done, pupils will be dilated using 1% atropine and corneal hydration will be maintained using methylcellulose.

After general surgery, including eye surgery if necessary, is complete, the animal will be paralyzed with pancuronium bromide (induction dose will be sufficient to induce paralysis as judged by a relaxing of eye muscles and cessation of breathing; maintenance dose of 0.2 mg/kg-hr) and artificially ventilated. Once ventilated, the chest will be opened and the heart exposed to facilitate injections of microspheres.

Intravitreal injections of pharmacological agents

In experiments requiring intravitreal injection of drugs, the method developed by Saszik and Frishman (Saszik et al. 2001) will be used. A small hole in the eye just behind the limbus will be made using a 30G needle. 1.5 μ l of solution containing the pharmacological agent will be injected into the eye through the hole using a glass pipette tip (~20 μ m) on a microsyringe. Initial doses of agents will be chosen to produce a vitreal concentration similar to what is currently used in cat and monkey (Robson & Frishman, 1995; Sieving, et al., 1994). For rats, a vitreal volume of 39 μ l will be assumed (Bush et al., 1995). To assess the efficacy of these agents, dark-adapted ERG responses will be monitored until the response stabilizes. If no change occurs in the ERG, the injection will be repeated.

Personnel and Qualifications

Regulations issued under the recently amended Animal Welfare Act require that "Personnel conducting procedures on the species being maintained or studied will be appropriately qualified and trained in those procedures..." In addition to the responsibility of the Principal Investigator to assure that those individuals conducting such procedures are properly trained and qualified, the Institute Animal Care and use Committee is required to evaluate the qualifications provided by the Principal Investigator in the course of ACUP review.

Descriptions of qualifications must include 1) formal education, 2) relevant general experience in the performance of procedures in the species studied, and, as applicable, 3) specific training and experience in the performance of non-routine, invasive, and surgical procedures in the species studied.

Research protocols should include only the names of the Principal Investigator and those individuals actually performing animal care and use procedures. Teaching protocols should include only the names of the Instructor and those employed to assist the instructor during the teaching exercise. Exclude the names and qualifications of those individuals with non-animal support functions associated with the research/teaching protocol. Descriptions of training and experience in the performance of specified non-routine, invasive, and surgical procedures, unless they will be performed by a Clinical Veterinarian, must be included. If training/CV not attached, describe the training and experience of each individual.

CV attached or on file
(include training)

Principal Investigator/Instructor: Jameel Ahmed

Co-Investigators/Instructors:

Technicians:

SECTION II. ANIMALS AND HUSBANDRY REQUIREMENTS

What genus, species, and strain of animal will be used? *Rattus norvegicus*

What sex distribution is required? male female both either

What will be the approximate age, weight or size of the animals used? 300

What is the maximum number of animals per study? 60 (current estimate, more may be requested in later years (please see section I explanation))

How many control groups are planned? 0

What is the maximum number of treatment groups per study (include controls)? 1

How many animals per treatment group? 60

Is after hours monitoring of animals required? Yes No

If yes, explain the circumstances that necessitate after hours monitoring and who will monitor.

What is the maximum number of days the diet will be fed?

What, if any, are the anticipated side effects?

Is dietary restriction required? Yes

If yes, what is the anticipated maximum duration (hours) of dietary restriction? 18 hours

How often will the diet be restricted (frequency)? once total

Is special housing required? no

If yes, describe housing requirements.

SECTION III. ROUTES OF ADMINISTRATION, TEST SUBSTANCES

Will a test substance be administered? Yes No

If no, go to Section IV.

If yes, indicate all routes of administration: Tetrodotoxin (TTX) will be delivered via injection into the vitreous after the animal is anesthetized.

Describe the dosing regimen, including maximum frequency and duration, for each route of administration:

TTX (injection volume of 1.5 µl) will be given during the experiment only. Effectiveness of TTX will be monitored by examining the electroretinogram for characteristic changes due to removal of spiking activity. TTX injection will be repeated until the effect on the electroretinogram is complete and stable. Appropriate dosage of TTX in the rat retina is not yet known and will be determined by starting with the dose that is appropriate for cat and mouse and altering this dose in later experiments.

SECTION IV. RESTRAINT

Will animals be restrained? Yes

If any test substance as described in Section III will be administered, will the method of restraint (for test substance administration only) be brief, routine restraint? Yes No

If yes, do not include a description of the restraint technique for test substance administration below.

If no, include a description of the restraint technique to be used for test substance administration below.

Test substances will be administered after the animal is anesthetized.

Describe the applicable methods of restraint for each procedure. The descriptions should include the restraint methods to be used for procedures such as examination, test substance administration (other than routine manual restraint), anesthesia administration, and euthanasia, as well as for procedures unique to this protocol.

Brief, routine manual restraint will be used for anesthesia administration. Test substances will be administered after anesthesia.

Chemical Restraint, non-surgical procedures. List procedure(s), the chemical agent including dose and route of administration, and anticipated duration and frequency.

SECTION V. EUTHANASIA

Will the animals be euthanized? Yes

If no, go to Section VI.

If yes, indicate method(s). If a method employs the use of a drug or chemical, indicate the name of the agent, dose and route of administration.

At the end of the experiments, euthanasia will be carried out using an overdose of sodium pentothal (5%) intravenously. Death will be ensured by monitoring blood pressure and ECG activity and by direct observation of the heart.

SECTION VI. NON-SURGICAL PROCEDURES

Is it anticipated that any of the non-surgical animal procedures conducted in the course of this study will cause pain or distress, other than that which is momentary or transient?

No

If no, go to section VII

If yes, identify each procedure and any measures taken to alleviate pain or distress pre-procedural, procedural, and post-procedural, including the use of anesthetics, analgesics and tranquilizers, or other measures such as behavioral conditioning.

If yes, and measures will not be taken to alleviate pain or distress, what is the rationale for not alleviating pain or distress?

Retrospective reporting of any unanticipated pain or distress that occurs in study animals for any reason is the responsibility of the Principal Investigator. This applies to surgical as well as non-surgical procedures, and includes painful or distressful effects of test substances. As soon as the animal portion of the study is completed, the Principal Investigator should submit a written report stating (a) the ACUP number, (b) the numbers and species of animals involved, (c) a description of the events resulting in unanticipated pain or distress, and (d) measures taken to prevent future occurrences of such events, and, should such events recur, measures to be taken to alleviate the pain or distress.

SECTION VII. SURGICAL PROCEDURES

Will surgery be performed? Yes

If no, go to Section VIII.

Who will perform the surgical procedures? Dr. Ahmed

Where will the surgical procedures be performed (building and room/lab numbers)?
M112

Will the surgical procedures be major? or minor?

Will survival surgical procedures be performed? No

All survival surgical procedures must be performed using aseptic technique.

If yes, will multiple survival surgical procedures be performed?

If multiple survival surgical procedures will be performed, provide an explanation of why this is necessary.

Will chronic instrumentation be performed? n/a

List dose and route of administration of each agent used as a(n):

Pre-anesthetic:

Anesthetic:

Post-anesthetic:

Will an analgesic be administered post-operatively? n/a

If yes, identify each analgesic to be administered and indicate the dose, route of administration, and frequency of administration.

If post-surgical pain or distress is anticipated, and an analgesic or other appropriate agent will not be administered, explain the necessity of withholding or the contraindications to the use of analgesics or other appropriate agents.

n/a

Who will monitor the animals during anesthesia?

Who will provide post-surgical care/monitoring?

Where will the animals recover from anesthesia?

Will any paralytic or neuromuscular blocking agent be administered?

If yes, indicate the agent, dose, and route of administration.

SECTION VIII. EVALUATION OF ALTERNATIVES TO THE USE OF ANIMALS AND PAINFUL PROCEDURES

A. Replacement Alternatives

Describe any alternatives to animal testing and/or painful procedures you considered for this protocol. The investigator is required to consult information sources such as Biological Abstracts, Index Medicus, Medline, Current Research Information Service, the Animal Welfare Information Center (National Agricultural Library), or other sources in an effort to identify possible replacement alternatives. List the information sources consulted and describe any alternatives to animal testing procedures you considered. If existing alternatives are unsuitable, explain. If the use of animal testing is required by regulations, cite the appropriate regulation and/or testing guideline.

A search for alternatives was done on the ALTWEB database. Search words included rats and retina and blood flow and (refine or reduce or replace). No alternatives were identified using this search.

B. Reduction Alternatives

Describe measures considered, such as appropriate statistical evaluations, or the use of *in vitro* modeling coupled with an *in vivo* component, that tend to reduce the total number of animals used.

Since the early parts of this project centers around development of a technique, it is difficult to anticipate the number of animals needed. Largely, this will depend on the inherent variability in the measurement technique. If the measurement technique has a high variability, more animals will have to be used to achieve statistically significant results. 20 animals have been requested, as this seems a reasonable number to work out technical problems and obtain baseline measurement. All attempts will be made to limit the number of animals used.

C. Refinement Alternatives (Minimization of Pain and Distress)

If painful or distressful procedures are a component of your procedure, what measures are taken to employ methods that tend to minimize the degree or duration of pain or distress, other than the administration of anesthetics, analgesics, or tranquilizers, such as 1) the minimization of time of exposure to a painful or distressful procedure or situation, 2) behavioral conditioning or modification, and 3) the use of available spontaneous animal models in lieu of induced animal models?

n/a

SECTION IX. RESEARCH DUPLICATION

(Skip Section IX if ACUP is for teaching purposes.)

Has this protocol been reviewed against data already in the literature to ensure that this study does not unnecessarily duplicate previous experiments? Describe your review.

NIH reviews of a previous incarnation of this grant proposal has suggested that the work is novel and interesting.

The following is an excerpt from the background and significance section of the proposal which describes how the proposed research will fill in a gap in current research.

The retinal circulation does an excellent job of matching the nutrient supply to the inner retina, which is largely supplied by the retinal circulation (e.g. Linsenmeier, 1986), with the metabolic demand of inner retinal cells. Perturbations to either the supply or demand sides of this mass balance result in compensatory changes in retinal blood flow. Figure 1 illustrates this mass balance concept

Changes in the supply side of the mass balance have been shown to cause changes in retinal blood flow. Hypoxemia has been shown to result in increased retinal blood flow (RBF) (e.g. Ahmed et al., 2001), while hyperoxia has been shown to decrease retinal blood flow (Grunwald et al., 1984).

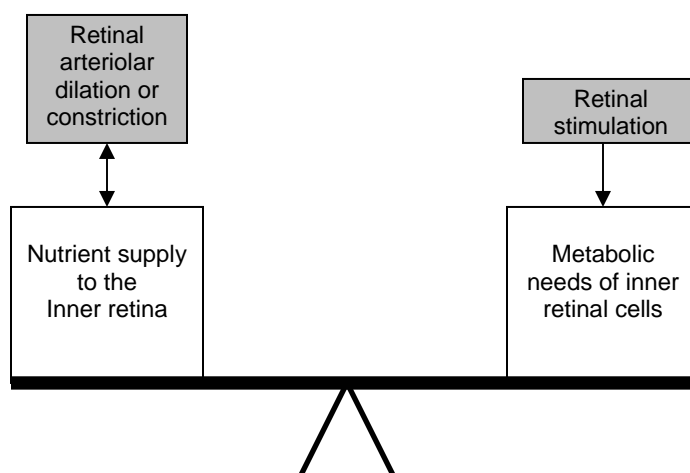


Figure 1: The retinal circulation works to balance the nutrient supply of the inner retinal with metabolic needs

Alteration in the oxygen consumption of inner retinal neurons also results in changes in RBF. Stimulation of the retina with flickering light, which strongly increases inner retinal oxygen consumption (Bill and Sperber, 1990), results in increased blood

flow to the optic nerve head, as measured by laser Doppler flowmetry (Falsini et al., 2002), as well as increased retinal vessel diameter (Polak et al., 2002). It has also been shown that flicker results in a transient decrease in oxygenation of ONH tissue (Ahmed et al., 1994). It has been shown that this increase in ONH blood flow is accompanied by an increase in NO levels in the vitreous above the ONH (Buerk et al., 1998).

While the link between oxygen supply and demand in the retina has been fairly well chronicled, the major components of the local control system underlying this link are less well understood. Figure 2 shows one of an infinite number of hypothetical control systems that might be in place to control retinal vascular tone, which is the parameter that controls retinal blood flow. This control system shows a number of candidate molecules that have been shown to exert a vasoactive effect in the retina and how they might interact to control retinal vascular tone. One of these substances is nitric oxide (NO) produced by both neuronal nitric oxide synthase (nNOS) and endothelial nitric oxide synthase (eNOS) (see Goldstein et al., 1996 for review of NO's role in retinal physiology). Also included in this hypothesis are other vasoactive compounds that have been shown to alter blood flow in the retinal circulation, including adenosine (Gidday and Park, 1993; Buerk et al., 2002), endothelin (Granston et al., 1990) and $[H^+]$ (Rassam et al., 1993). Also shown in this figure are hypotheses for how alterations in the oxygen supply or demand of the inner retina (represented by dark boxes) might result in changes in release of vasoactive compounds. It should be noted that the hypothesis represented by figure 2 is largely untested (solid arrows indicate connections which have significant evidence supporting them, dashed arrows indicate hypothetical relationships). To prove any of these interactions, it is not only necessary to show that exogenous administration of these test substances results in a change in retinal blood flow, but also to show that blockage of the effect of these substances results in an inhibition of normal physiological function.

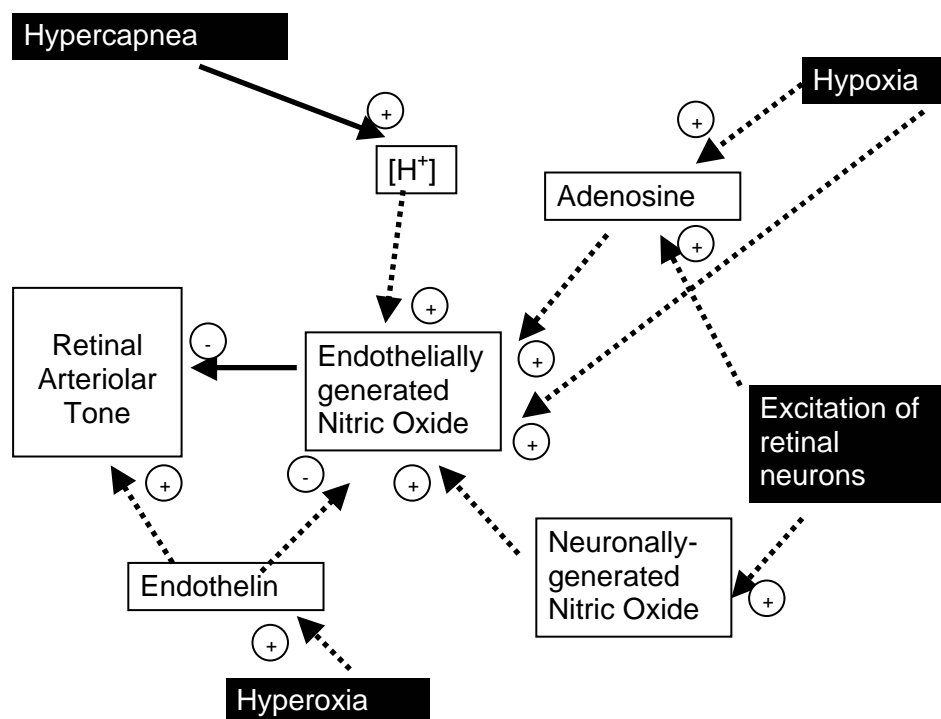


Figure 2: Hypothetical control system for retinal blood flow: Dashed arrows indicate hypothetical functional relationships, while solid arrows indicate well established functional relationships. Dark boxes indicate perturbations that have been shown to alter retinal blood flow.

There is evidence that each of the candidate molecules shown in figure 2 can alter retinal vascular resistance, however, it is not at all clear which of these plays an important role in normal physiological function. To be able to perform the necessary experiments to pick apart this control system, it is necessary to develop an animal model in which accurate retinal blood flow measurements can be made and in which the administration of test substances can be done easily.

There are numerous questions about functional interactions between neurons and the retinal vasculature that remain to be answered. The question addressed in specific aims #3 and #4 deals with developing an understanding of the mechanism by which flicker stimulation of the retina results in dilation of retinal vessels. The origin of this signal must be one of the cell types that are present in the inner retina. These cells include the numerous subclasses of horizontal cells, bipolar cells, amacrine cells, and ganglion cells (including both cell bodies and axons), as well as Müller cells and astrocytes. In particular, specific aim #4, in which TTX will be used to block spike-generating activity in retinal amacrine and ganglion cells, is designed as a start in identifying the neuronal trigger signal that ultimately leads to vasodilation.

One possibility for the neuronal signal is simply to use aggregate action potential generation in ganglion and amacrine cells as a measure of inner retinal excitation. If this is the case, blockage of action potential generation with TTX will result in a severing of the link between retinal excitation and retinal blood flow. If TTX proves to have little or no effect on flicker-evoked vasodilation, than it would mean either that the signal that initiates this response occurs more distally in the retina, that the signal used is an overall integration of all inner retinal activity, perhaps integrated via retinal glial cells, or some combination of these two.

The responsible Principal Investigator affirms that the study design, for which the above procedures will be performed, represents his/her best efforts for the reduction of numbers of animals used in this research, including the consideration of alternatives to the use of animals, and for the minimization of pain/distress in research animals. Furthermore, the experience of the individuals listed in the qualifications section have, individually or collectively, sufficient education, training, and experience to perform all of their assigned portions of the described animal care and use activities. The Principal Investigator will review and reevaluate the qualifications and training of all personnel involved in these animal care and use activities on at least an annual basis.

Principal Investigator

Date

THIS SECTION TO BE COMPLETED BY VETERINARIAN

The Veterinarian has reviewed the proposed animal care and use procedures and has found them appropriate for this protocol.

Attending Veterinarian

Date

ATTACHMENT B

REVIEW AND EVALUATION FORM

ACUP REVIEW AND EVALUATION RECORD

ACUP No: _____ **Date:** _____

Title: _____

ACUP Review Subcommittee: _____

Primary Reviewer: _____ **Secondary Reviewer:** _____

Subcommittee review and evaluation of this submission included but was not limited to consideration of the following: (all may not apply)

The Principal Investigator responsible for this ACUP has offered written assurance that where applicable:

1. The species selected is considered appropriate for this procedure.
2. The experience and qualifications of the Principal Investigator and Laboratory Assistants are appropriate to perform this activity.
3. Living conditions will be in accordance with existing regulatory standards.
4. Minimum number of animals are to be used consistent with the needs of the procedure described.
5. Painless euthanasia will be provided if needed.
6. Pain, discomfort, and distress will be minimized.
7. More than momentary or slight pain will be appropriately eliminated or minimized or will continue only for the necessary period of time.
8. Animals experiencing chronic or severe unrelieved pain will be euthanized during or immediately at the end of the procedure.
9. Appropriate surgical services and pre- and post-operative veterinary and nursing care will be provided.
10. Multiple survival operative procedures will not be performed unless appropriate justification is provided.
11. A literature search was conducted and non-animal alternatives were either not available or considered not appropriate.
12. This activity does not unnecessarily duplicate previous experiments. (Research only)
13. Medical care is available and will be provided by a qualified veterinarian.

Action Approved

Require Modification(s)
(List)

Approval Withheld

Reviewer

Date

ATTACHMENT C

NOTIFICATION FORM

NOTIFICATION TO INVESTIGATOR OF ACUP REVIEW

To: _____ (*Principal Investigator*)

ACUP No. _____ has been reviewed by the Institutional Animal Care and Use Committee (IACUC), and the following action was taken:

- Approved
- Approval Withheld
- Requires Modification

If this ACUP requires modification(s), this is described below. All changes should be discussed with the primary reviewer listed below, or in case they are unavailable, with the secondary reviewer.

REQUIRED MODIFICATION(S):

Upon modification, please resubmit the completed ACUP. No experiment can be initiated for this ACUP until the Principal Investigator has received written notification of the ACUP's approval.

Signed: _____

Date: _____