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CONTRACTING ORGANIZATION: Rutgers Biomedical and Health Sciences

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14. ABSTRACT

Blast-related injuries can result in retinal detachment (RD). RD causes separation of the neural retina from the retinal pigmented epithelium and uncouples photoreceptors from their synaptic partners, ultimately leading to blindness. We discovered that synaptic damage is due in part to an increase in the activity of Rho kinase (ROCK). We proposed therefore to prevent the disruption to retinal synaptic circuitry after injury by using a highly efficacious ROCK inhibitor, AR13503. The work is done on adult pigs, whose retina is similar to humans, to increase the translational potential of the results. Our results on the protection of rod synapses with AR13503 have been published. This year we primarily focused on cone synapses. We found that cone ribbons shorten dramatically after detachment and that synaptic invaginations flatten. These disruptive changes can be prevented with ROCK inhibition by intravitreal injection of AR13503. The drug is effective whether applied at the time of detachment or after a delay of 2 hrs. Thus it could be applied at the time of an iatrogenic detachment, as done for gene therapy or stem cell transplants, as well as shortly after a blast injury. After reattachment, cone synaptic morphology appears normal however ERG responses for cone vision are abnormal. Application of drug improves function. ROCK inhibition protects both rod and cone synapses from injury over time and could help preserve vision for individuals who suffer trauma.

15. SUBJECT TERMS

Retinal detachment, Rho kinase, synaptic disjunction, rod and cone photoreceptors

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Report Outline

1. INTRODUCTION

2. KEYWORDS

3. ACCOMPLISHMENTS
   - What were the major goals of the project?
   - What was accomplished under these goals?
   - What opportunities for training and professional development has the project provided?
   - How were the results disseminated to communities of interest?
   - What do you plan to do during the next reporting period to accomplish goals?

4. IMPACT
   - What was the impact on the development of the principal discipline(s) of the project?
   - What was the impact on other disciplines?
   - What was the impact on technology transfer?
   - What was the impact on society beyond science and technology?

5. CHANGES/PROBLEMS
   - Changes in approach and reasons for change
   - Actual or anticipated problems or delays and actions or plans to resolve them
   - Changes that had a significant impact on expenditures
   - Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

6. PRODUCTS
   - Publications, conference papers, and presentations
   - Website
   - Technologies
   - Inventions
   - Other products

7. PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS
   - What individuals have worked on the project?
   - Has there been a change in the active other support of the PD/PIs or senior /key personnel since the last reporting period?
   - What other organizations were involved as partners?

8. SPECIAL REPORTING REQUIREMENTS

   - Quad Charts

9. APPENDICES

1. INTRODUCTION

Our research is directed toward preventing trauma-induced visual loss. Eye trauma is the 4th most common injury in combat. In blast-related injuries, the most common cause of ocular damage, the retina frequently is detached from its underlying supportive pigment epithelium. This injury results in the disjunction, or impairment, of the synapses between the rod and cone cells and their postsynaptic bipolar cells. The loss of the first synapse in the visual pathway necessarily results in visual loss. We reported that detachment causes rod synaptic disruption very quickly (within 2 hours). Moreover, we discovered an approach that can significantly reduce the loss of synaptic connectivity at the first synapse. By reducing the activity of Rho kinase (ROCK), we can reduce trauma-induced cytoskeletal changes in photoreceptors and decrease the extent of rod synaptic terminals retraction and separation from their postsynaptic partners. Moreover, this inhibition of rod synaptic disjunction is correlated with improved scotopic electroretinographic (ERG) responses. This past year we continued our previous year's focus on the effects of injury to cone synapses after detachment and rescue from injury with ROCK inhibition. We are using adult pigs so that our results have the potential for translation to human patients.

2. KEYWORDS

Retinal detachment, retinal reattachment, rod photoreceptor, cone photoreceptor, rod spherule, cone pedicle, synaptic ribbon, synaptic retraction, synaptic disjunction, Rho A, Rho kinase, Lim kinase, ERG, scotopic or photopic responses, confocal microscopy, trauma

3. ACCOMPLISHMENTS

-What were the major goals of the project?

The major goals as stated in the SOW were the following:

Specific Aim 1- Test the ability of the new ROCK inhibitor netarsudil-M1, AR-13503, to stabilize photoreceptor synapses via single injection and/or a sustained–release delivery system over several days

Milestone #1 Determination of the dose response curve for soluble AR-13503- 100% complete
Milestone #2 Direct determination of whether a sustained-release drug application is an improvement over a single injection- 100% complete
Milestone #3 Assessment of an expansion of the length of time over which the injured, detached retina can be protected with the ROCK inhibitor AR-13503- 70% complete

Specific Aim 2- Determine how long after a retinal detachment injury a drug can be applied and still reduce synaptic disruption

Milestone #4 Determination of effectiveness of ROCK inhibition in a delayed treatment- 95% complete

Specific Aim 3- Determine if drug (either a ROCK or LIMK inhibitor) at the time of surgical reattachment helps promote recovery

Milestone #5(previously #7 in SOW) Determination of the efficacy of a drug application as an adjunctive treatment with retinal reattachment surgery-10% complete

-What was accomplished under these goals?

We have published the procedures, results and analysis on the effects of retina detachment on rod synapses and the ability of ROCK inhibition using AR13503 to dramatically reduce injury to rod synaptic transmission. In addition to the finding that ROCK inhibition prevents injury, we reported that injury occurs very rapidly, within 2 hrs of detachment, and that the injury, in the form of synaptic disjunction, spreads throughout the retina, including areas that are not detached. This past year we built on preliminary data about the effect of detachment and rescue via AR13503 of cone synapses, looking at early time points and possible spread of injury in the retina.

An initial challenge to the accumulation of data on cone synapses was the fact that not all our specimens survived the long periods in the freezer made necessary by the pandemic when physical presence at the University for employees was prohibited unless they had clinical responsibilities. In some cases, dehydration and perhaps oxidation had occurred ('freezer burn' in layman's terms). Although at low magnification the specimens appeared to be in good condition, at the higher magnifications needed for the analysis of synaptic morphology, the variability in the penetration of the immunohistochemical labeling was poor, and, thus, for selected specimens we did not feel confident in the results. We have now repeated all the experiments we felt were necessary to obtain optimally preserved material. The experiments were replicas of our previous experiments: Both eyes receive a detachment in the inferior nasal quadrant, drug dissolved in balanced salt solution (BSS) is injected into one eye, BSS into the other, and then detachments remain for an additional 2-4 hours or 2 days before euthanasia and enucleation. We used doses of the drug AR13503 which we had previously shown were efficacious for protection of rod synapses and compared synaptic damage in the treated eye with damage in the untreated eye.

Milestone #1:

To examine cone synapses, retinal sections were triple-labeled for PSD-95, which labels all presynaptic terminals, CtBP2, which labels all synaptic ribbons, and peanut agglutin (PNA) which is specific for cone cells. Fluorescently tagged PNA labels the membrane of the entire cone cell but is particularly prominent at the outer and inner segments and the cone pedicle. In this way, we could distinguish cone from rod synapses. Sections were examined either with conventional confocal microscopy or stimulated emission depletion (STED) microscopy. In both cases, stacks of 40 (conventional confocal) or 160 (STED) images, 2-4 stacks for each microscopical technique, were used for 3-D reconstruction. The length and shape of synaptic ribbons as well as the overall all size and shape of the pedicles could therefore be assessed.

Normal retina. We examined 2 normal animals, euthanized in the same way as experimental animals but with no surgical intervention. In the normal retina, pedicles are geometrically arranged in the outer plexiform layer (OPL) above/vitread to the rod spherules. Based on measurements of PNA label along the base of the pedicle, they average 25 um^2 in area, at the point where cone terminals meet the second order neurons. Each pedicle contains on average 11 ribbons and the ribbons are arched, looking like capital "u's". Since the ribbons are close to the surface of the synaptic invaginations at the base of the pedicle, ribbon curvature is an indication of the depth of the invagination. If there is no invagination, the

ribbons, assuming they remain close to the presynaptic membrane, would be flattened (see Quad Chart image).

Two-hour survivals. Using older material from 2 animals and one animal from a new experiment, we found that cone pedicles in retinas that had suffered a 2-hour detachment showed several changes. First, the length of the ribbons shortened compared to the normal retina (Fig. 1A-C; all figures are presented after the research information and before the Impact statement); second, the degree of arching was reduced (I.e., the ribbon architecture changed from an arched to a flattened appearance): and third the number of ribbons in some pedicles was reduced suggesting that some ribbons are lost. Thus, like rod spherule changes, cone pedicles are damaged rapidly. However, the damage does not spread to attached (uninjured) parts of the retina, as is does for the damage to rod spherules.

0.75 uM AR13503 injected intravitreally at the time of detachment reduced damage to the cone synapses. For ribbon length, the reduction was statistically significant. Changes in ribbon arching were qualitatively assessed; the change in arching was easily noted with STED 3-D reconstructions which have a resolution of about 50 nm (Fig. 2). In contrast, rod ribbons showed no difference in length or changes in curvature, in treated versus untreated retinas (Fig. 1D). Finally, the size of the base of the pedicles decreased in the treated retina, compared to the untreated retina and compared to normal retina.

Overall, AR13503 protects many structural aspects of cone pedicles after detachment. One intriguing finding was that the size of pedicles *decreased* with ROCK inhibition, compared to untreated retina. The shape of cone pedicles has been reported by Steve Fisher and colleagues to change with detachment by rounding and flattening and it is possible that ROCK inhibition locks this change in place at the beginning of the injury. The preservation of arching in ribbons suggests that invaginations are prevented from flattening in treated retina further suggesting that AR13503 inhibits shape change.

(Please note, the optimal dose for rod synapses was 0.5 uM, although 0.75 uM was also effective in some eyes. In additional experiments, described below, we used retina treated with 0.5 uM AR13503 to examine cone synapses.)

Milestone #2: See prior year annual report.

Milestone #3:

*Two-day survivals***.** To examine longer term effects of the ROCK inhibitor, we had previously examined eyes with detached retinas 2 days after the detachment injury. One eye was treated with a subretinal injection of 0.5µM AR13503 dissolved in BSS, and the other eye with BSS. By 2 days after surgery, most retinas had spontaneously reattached, so we could also look at retinal function with ERG.

The morphology and function of rod synapses, determined by confocal microscopy and scotopic ERG responses, respectively, were improved with subretinal injection of the ROCK inhibitor AR-13503. In addition, the anatomy and function correlated with one another, i.e., more synaptic disjunction resulted in lower scotopic responses. These results have been published.

Using material from one older experiment and 2 new experimental animals, we found that most anatomical changes to cone pedicles were normal after reattachment. In other

words, ribbon length and number appeared normal with no difference between treated and untreated eyes. The size of pedicles at their base was also not different between treated and untreated retinas; however, the base was still somewhat smaller for treated retinas than normal. There was considerable variability in the degree of ribbon arching: In some reconstructions there was shallow arching of the cone ribbons and in other stacks flattened ribbons were observed. Thus, the terminals did not look completely normal, although there was not a consistent difference between treated and untreated retina.

As previously reported, we also looked are cone photoreceptor function after spontaneous reattachment of detached retina. Since the ERG recordings remain useful from the older experiments, we have been able to examine cone-specific responses from the same animals (n=5) that we used to examine rod-specific, scotopic responses. Because there can be considerable variability in responses between eyes in the same animal, we assess changes over time compared to the original baseline in each eye individually. For photopic flicker responses, which test cone function primarily, we did not observe any statistical difference in eyes treated with ROCK inhibition compared with those treated with BSS. However, the data were very variable. In other words, some animals (3 of 5) did show improved function by 14-32% in the treated eye compared to the untreated eye. However, for the photopic b-wave (0 dB) responses, which also test cone function primarily, we did find a small but significant increase in the amplitude of the response in the eyes treated with the ROCK inhibitor compared to the untreated eye (GEE statistics). The difference in the photopic b-wave was not due to transduction differences as the a-waves were no different for treated and untreated retinas (Fig. 3).

In summary, some morphology of the cone pedicles appeared normal after reattachment, although ribbon curvature was not. But there was no obvious morphological correlate for improved function of cones in treated retinas.

Seven-day survivals. To look at longer time periods after treatment of a single subretinal injection of AR13503, we maintained pigs for 1 week after detachments were made. Again, we treat one eye with the drug dissolved in BSS and the other with BSS. The animals are examined with ERG, making baseline recordings before any surgery and then again at 7 days. After the day-7 ERG recording and examination by fundus photography and OCT, the animal is euthanized and enucleated. In the past year, we did 2 new experiments with one-week survivals. Both animals had inflammation in the eye after the one-week period; their data, therefore, was not used.

We also examined ON bipolar cells in a 1-wk survival animal whose retina had not reattached. Using both a Go alpha and a PKC alpha antibody we detected bipolar sprouting into the outer nuclear layer of both rod and cone bipolar dendrites. Such sprouting has been previously described by Sakai et al. (2013). Although more attention has been paid to neurons postsynaptic to rod cells, these data and the work of Sakai et al. suggest that neurons postsynaptic to cone cells are also affected by detachment.

Milestone #4: We have examined delayed treatment with ROCK inhibitor after detachment. We used a delay of 2 hours. Both eyes undergo BSS-induced retinal detachment; 2 hours later drug is applied by intravitreal injection of 0.5µM AR13503 into one eye and BSS into

the other eye. After 2 more hours the animal is euthanized and enucleated, and the retinas are examined for synaptic damage.

For rod synapses, we have previously reported analysis of data from 5 animals. The result was significant (GEE and with mixed random effects statistics). Synaptic disjunction of rod synapses was reduced by 35.3% by intravitreal injection of 0.5uM AR13503.

We have examined 4 animals, 2 from previous experiments and 2 new experiments, for the response of cone synapses after a delayed treatment. The results suggest that ROCK inhibition provides some protection for cones. Eyes treated with AR13503 had generally longer ribbons. In untreated eyes, ribbons were generally smaller and, with both conventional confocal microscopy and STED reconstructions, we observed some pedicles completely devoid of ribbons. The statistical analysis of treated versus untreated cone pedicles has recently been completed (Fig. 4). However, we also observed that many ribbons were completely flattened in the untreated retina, indicating the loss of synaptic invaginations, whereas the treated retina showed ribbons with shallow arching (Fig. 5). Additionally, the base areas of pedicles were smaller than normal for both treated and untreated retina, although the treated retina still had pedicles smaller than those in untreated retina. Thus, 4 hours of detachment can be very damaging for cone ribbons and synaptic invaginations. It appears that AR13503 can prevent some of this damage.

These results are exciting, as they suggest that treatment of patients with a ROCK inhibitor that is delayed by a few hours after injury will provide some protection to photoreceptors. Delayed treatment will be important for translation to clinical practice, as it will allow for treatment of soldiers and civilians who cannot be treated immediately after injury.

Milestone #5: See previous annual report.

Summary. The work of the past year, combined with our previous work, demonstrates that cone pedicles and rod spherules respond in unique ways to retinal detachment. Cone pedicles have changes in size, in depth of synaptic imaginations as seen by flattening of ribbons, and in number and length of ribbons. Rod spherules have no changes that we observed in curvature, number or length of ribbons. The spherules however, retract away from their postsynaptic partners and withdraw to the outer nuclear layer. Cone and rod cells are known to have many molecular differences; differences in their injury responses adds to this diversity.

Rod spherule retraction occurs in detached retina but also throughout the injured eye even if the retina remains attached. In contrast, the cone pedicle changes that we observed were not present in attached retina.

The long term (2 day) effects after reattachment are also diverse. Both cone and rod cells have reduced b-wave amplitudes after reattachment. Many spherules remain retracted after reattachment, which correlates with the functional loss of rod vision. In the pedicles, ribbon number and length appear normal after reattachment. Ribbon morphology, however, is not back to normal and pedicle size of treated retina is smaller than normal. These abnormalities, however, are not obviously related to the loss (or gain) of function. Rather it seems possible that, like several retinal degenerations, there are changes in the cone ON bipolar cells that may reduce synaptic transmission. Candidates are mGluR receptor reduction

or changes in the Go alpha signaling cascade. Nonetheless, a single injection of our ROCK inhibitor, whether at the time of injury or a few hours later, reduces morphological damage for both cone and rod synapses and improves function, as seen with ERG recordings.

***Traumatic brain injury (TBI) in mice*.** We have obtained some additional grant funding to look at retinas of mice with traumatic brain injury**.** In our DoD grant, we proposed that ocular problems, associated with 70% of TBI patients, are due to retinal damage similar to what we observe with retinal detachment. We are now pursuing this hypothesis with this increased grant support.

-What opportunities for training and professional development has the project provided?
Nothing to report

-How were the results disseminated to communities of interest?

The PIs and the postdoctoral fellow (who is currently a consultant) supported by this grant are all members of the Association for Research in Vision and Ophthalmology. We will submit an abstract for the annual ARVO meeting and hope to present our work in the spring.

This past year, an abstract was submitted and accepted for the Vail Vitrectomy meeting, which is an invitation-only conference. Some of our results on cone synapses were presented there by Marco Zarbin. We were also invited to review our work at an ARVO symposium focused on translational science. Drs. Townes-Anderson and Zarbin presented the work. Finally, a poster was presented at a New Jersey Meeting on Core Facilities. We presented some of our latest STED microscopic images; the poster received an award for excellence.

-What do you plan to do during the next reporting period to accomplish the goals?

We will work to complete our manuscript on cone synapses. This will entail completing all statistical analyses, making a few more renderings of pedicles, selecting figures, and finalizing the text of the manuscript.

We will also focus on completing data for analysis of 1-week survivals. This will mean 1-2 animal experiments and accompanying imaging and ERG recording. We hope to complete a manuscript of this work as well.

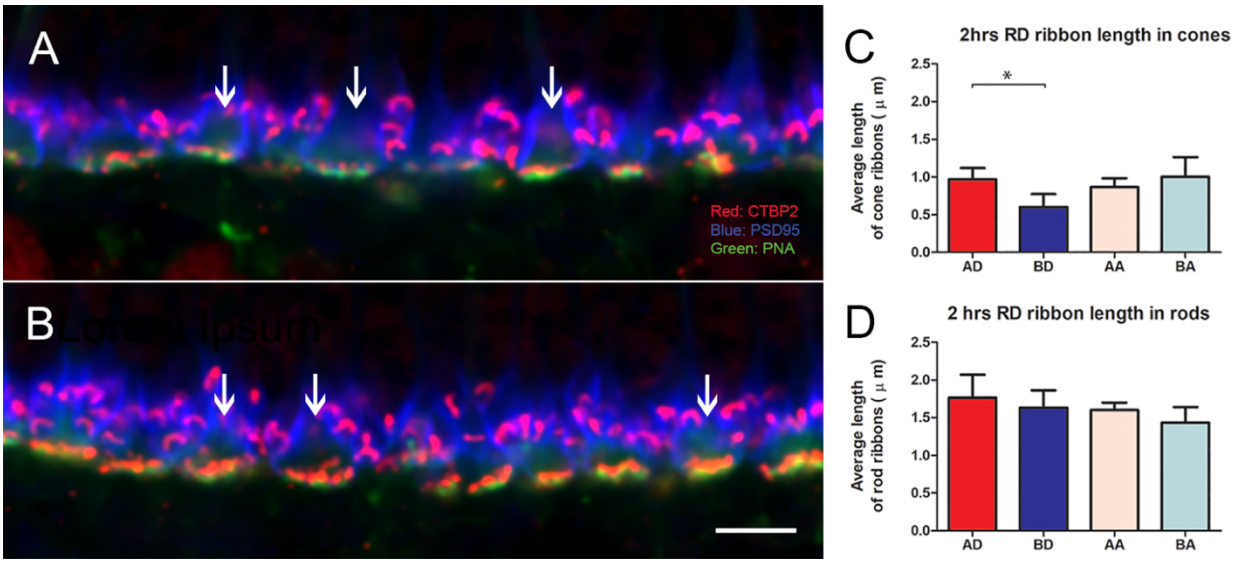

Fig. 1 **Two hours after detachment**. A. Detached retina, untreated. Although ribbons are present in most pedicles the arrows indicate pedicles with reduced and shortened ribbons. B. Detached retina treated with AR13503. All pedicles have ribbons (arrows). C. Length of cone ribbons decreased significantly in the untreated retina (BD) compared to the treated retina (AD), *p<0.0001. There was no significant difference in ribbon length in attached retinas whether treated or not (BA vs. AA). n=3 animals, mean ± s.d. D. Length of rod ribbons showed no differences in the treated vs. untreated eyes, in detached and attached retinas. n=3 animals, mean ± s.d. Bar=5 μm

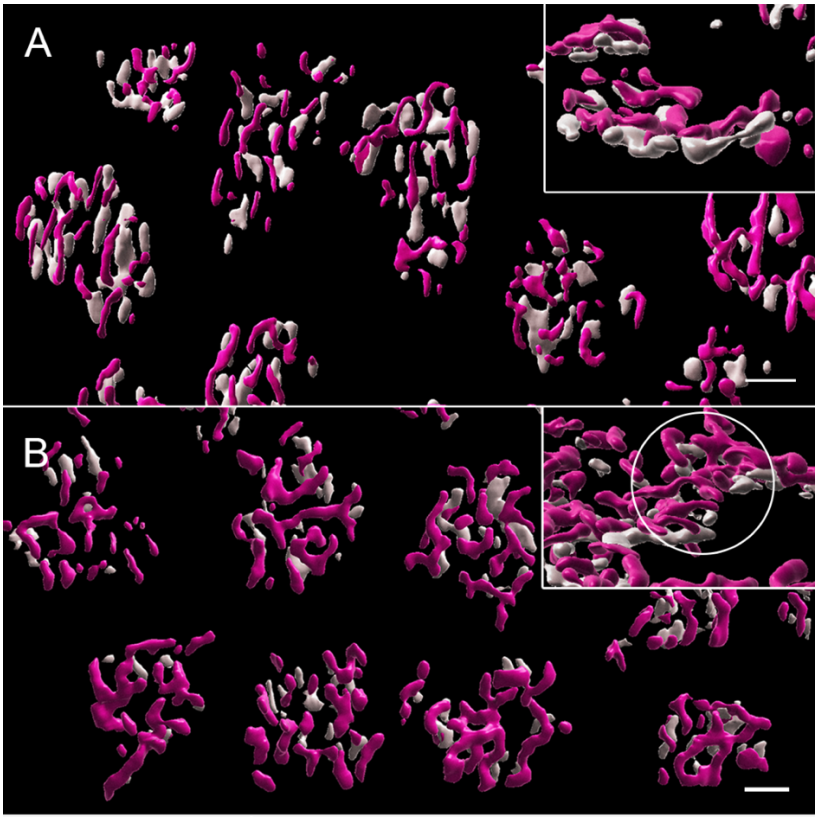

Fig. 2 **Two hours after detachment**. STED microscopy with surface rendering. A. Detached retina, untreated. The array of pedicles, highlighted by PNA label (gray), is less organized than the normal retina. The network of ribbons (pink) also looks less dense indicating a loss of ribbons. Branched ribbons were uncommon. In many cases, the ribbons have lost their horseshoe curvature (inset). B. Detached retina, treated. The array of pedicles looks more normal as does the network of ribbons overlying the PNA aggregates. In many cases, the ribbons have the characteristic u-shape curvature (inset, circle). Bars=2μm

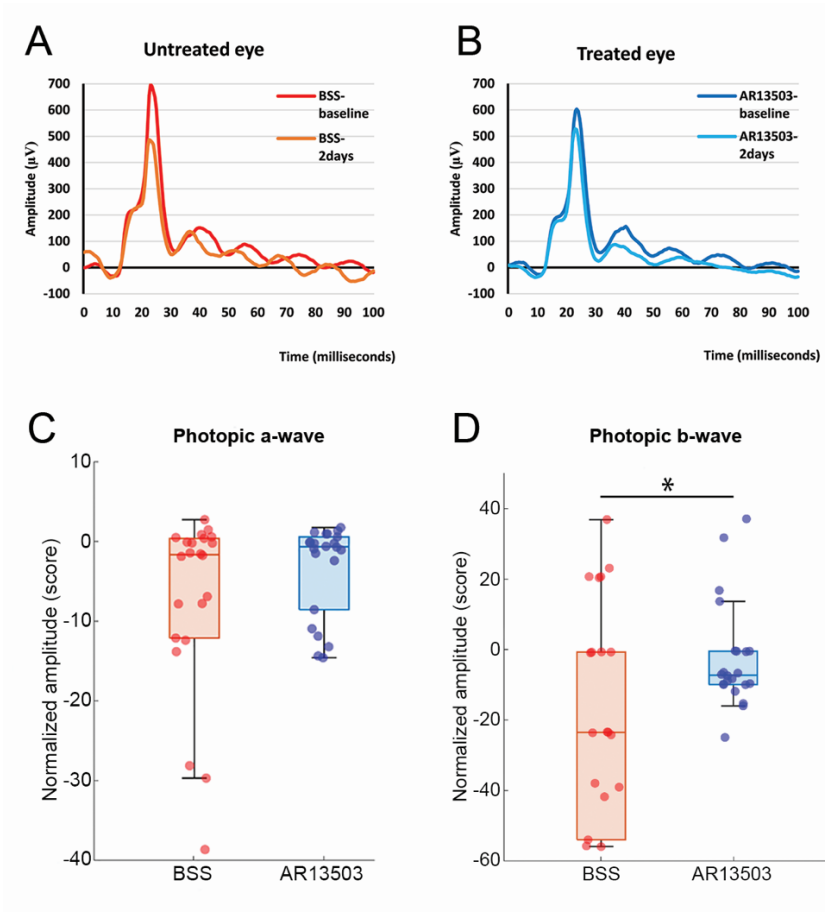

Fig. 3 **ERG recordings two days after detachment and with reattachment.** A, B. Representative waves of cone photopic b-wave for a BSS-untreated eye and an AR13503-treated eye. C. Forest plot showing normalized amplitude of photopic a-wave. There is no significant difference in a-wave amplitudes at two days from baseline recordings for both untreated and treated eyes. D. Forest plot showing normalized amplitude of photopic b-wave. BSS-untreated eyes have lower amplitudes at 2 days compared to baseline than AR13503 treated eyes, *p=0.0004, n=5 animals, average of 5 recordings, repeated 3 times per animal.

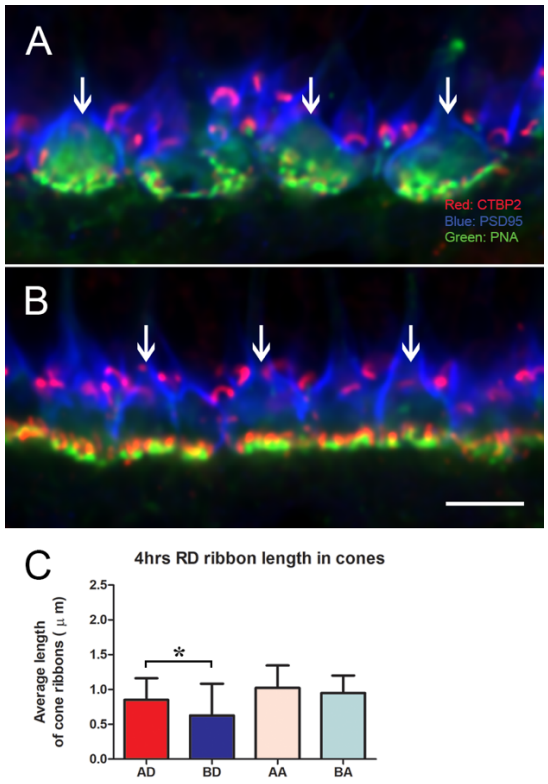

Fig. 4 **Four hours after detachment with delayed treatment.** A. Detached retina, untreated. Pedicles are rounded in shape (arrows) and ribbons are short. B. Detached retina treated with AR13503 after a two-hour delay. Pedicles have the more usual triangular shape (arrows) and ribbons appear longer. C. Length of cone ribbons in the untreated detached retina (BD) is significantly smaller than those in the treated retina (AD), *$p<0.05$. Length of ribbons in attached, untreated and treated, retinas (BA vs. AA) showed no significant differences. n=4 animals, mean ± s.d. Bar=5μm

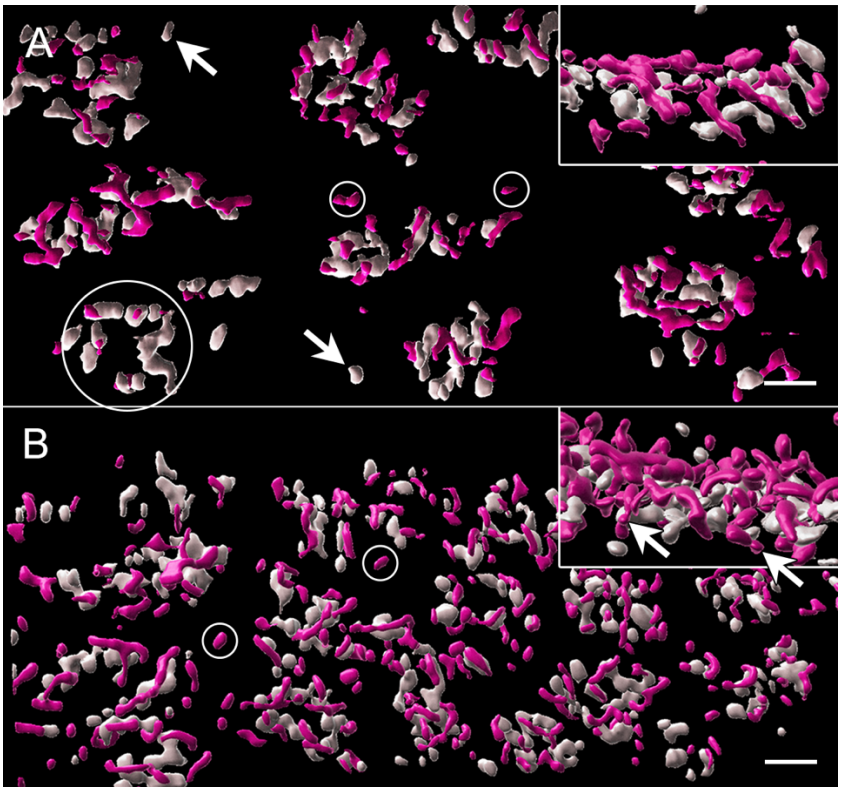

Fig. 9 **Four hours after detachment with delayed treatment.** STED microscopy with surface rendering. A. Detached retina, untreated. PNA aggregates (gray) have reduced numbers of ribbons (pink) which appear generally shorter than normal and in some cases thinner. Some pedicle bases are nearly devoid of ribbons (circle). Isolated PNA label (arrows) and ribbons (small circles) are present. Many ribbons are flattened against the pedicle base (inset). B. Detached retina, treated. Pedicles appear more tightly arrayed than in the untreated retina. There are more ribbons associated with PNA label but also some isolated ribbon fragments (circles). Some ribbons are arched, and many have "knobs" along their lengths and at their ends (inset arrows). Bars=2μm

4. IMPACT

-What was the impact on the development of the principal discipline(s) of the project?

Our work, both over the past three years and previously, leading up to the award of our grant, has demonstrated 3 things that are truly novel for the field of retinal trauma and care. First, injury to the synaptic circuitry of the retina occurs very rapidly after retinal detachment, within 2 hours. This observation applies to the rod synapses as well as to the cone synapses. The

rapidity may indicate a new urgency in how fast retinal detachment should be treated. Additionally, it may place retinal detachment on par with other central nervous system traumas such as spinal cord injury and stroke, which are now treated within hours. Second, our results show that for rod vision, retinal injury by detachment is not confined to the detachment but occurs all over the retina, i.e., in attached retina as well. Thus, the injury is larger than its gross manifestation and emphasizes the potential importance of rapid treatment. And third, we have demonstrated that a ROCK inhibitor, AR13503, is effective in reducing rod and cone synaptic injury if applied a few hours after or during detachment.

The practical application of this demonstration would be the use of ROCK inhibition for iatrogenic detachment used for gene therapy and transplantation of cells or implants. But additionally, we now are suggesting that delayed treatment would apply to retina trauma suffered on the battlefield or in accidental trauma.

-What was the impact on other disciplines?

It seemed possible to us that the RhoA pathway is involved in other traumas of the central nervous system and thus that ROCK inhibition might be useful for treating traumatic brain injury, for instance. Indeed, others have now published on this idea and confirmed our suggestion. But we also reasoned that the visual abnormalities seen with TBI may be due to synaptic disruption in the retina after brain injury. We had and now have more evidence that TBI does produce synaptic retraction in the retina and, thus, disjunction of the rod synapses. Thus, our studies on retinal detachment impact brain and visual injury more broadly.

-What was the impact on technology transfer?

Nothing to report

-What was the impact on society beyond science and technology?

The possibility of new therapies for ocular trauma should improve the quality of life for those who experience these traumas by reducing visual loss.

5. CHANGES/PROBLEMS

-Changes in approach and reasons for change

We have used STED imaging to better understand morphological change in photoreceptor synapses. The STED microscope available at RBHS is one of only 2 in the country and we were not certain initially that it would give us useful data. However, the ability to see cone ribbons, and the base of the cone pedicle, with PNA labeling, has given us a new perspective on the cone synapse and allowed us to observe changes, such as changes in ribbon arching, otherwise unobservable with conventional confocal microscopy.

-Actual or anticipated problems or delays and actions or plans to resolve them

As previously mentioned, there have been several changes and adjustments to the personnel working on the grant. Most recently Dr. Qian Sun has found a new job. Fortunately, she is willing to continue to do our immunocytochemistry and assist with animal surgeries as a paid consultant. Sectioning of the retina and imaging of the retina is done at the medical school core facilities by Luke Fritzky, Director of the Core, with oversight by Ellen Townes-Anderson. Eva Halasz continues to work on the projects and is critical to image and ERG analyses, which are done digitally and therefore can be accomplished long distance. Ilene Sugino works with Ellen Twones-Anderson on ERG recordings and imaging and also assists with surgeries. All members of the team stay in touch via the internet and zoom meetings are held periodically. Thus, we feel our personnel situation works well, and we are enthusiastically completing various aspects of the grant.

-Changes that had a significant impact on expenditures

Nothing to report.

-Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report

6. PRODUCTS

Publications, conference papers, and presentations

Impact of Rock Inhibition on Morphological and Functional Changes in the Cone Synapse After Retinal Injury. MA Zarbin, E Townes-Anderson, E Halasz, I Sugino, & L Frishman. Vail Vitrectomy 2022. March 12, 2022, Vail, Colorado.

Inhibition of photoreceptor-bipolar synaptic disjunction using pharmacotherapy: Implications for management of retinal detachment and other forms of CNS trauma MA Zarbin & E Townes-Anderson, ARVO, Tues May 3, 2022, Denver, CO

Use of advanced microscopic techniques at NJMS Cellular Imaging and Histology Core Facility to evaluate drug therapy for improved vision
L Fritzky, F Yousufzai, I Sugino, E Halasz, M Zarbin, & E Townes-Anderson, New Jersey State Core Facilities Conference

Website- Nothing to report
Technologies- Nothing to report
Inventions- Nothing to report
Other products- Nothing to report

7. PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Ellen Townes-Anderson
Project Role: Principle Investigator
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: 6
Contribution to Project: Supervisor of experiments, analyses, and publications, also participates in ERG recordings and oversees histological sectioning and imaging at the medical school's core imaging facility

Name: Marco Zarbin
Project Role: Co-Principle Investigator
Researcher Identifier): 0000-0002-7811-7132
Nearest person month worked: 1
Contribution to Project: Performs animal surgeries, reviews data analyses and publications

Name: Eva Halasz
Project Role: Postdoctoral Fellow, now Consultant
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: 4
Contribution to Project: Performs histological and ERG analyses, and participates in development of experiments and publications

Name: Qian Sun
Project Role: Research Teaching Specialist
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: 1
Contribution to Project: Surgical assistant in animal surgeries, maintains surgical equipment, harvests eyes after euthanasia, and performs immunocytochemical staining

Name: Ilene Sugino
Project Role: Research Interventionist
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: 1
Contribution to Project: Assists in surgeries, in ERG recordings, and in OCT and Fundus photography

Name: Amy Davidow
Project Role: Statistician
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: 1
Contribution to Project: Involved in all statistical analyses

Has there been a change in the active other support of the PD/PIs or senior /key personnel since the last reporting period?

Nothing to report

What other organizations were involved as partners?

Organization name: University of Houston College of Optometry
Location: Houston, Texas
Contribution: Collaboration with Dr. Laura Frishman

8. SPECIAL REPORTING REQUIREMENTS

Quad Charts- attached

9. APPENDICES