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14. ABSTRACT

The eye is an exposed organ that is particularly vulnerable to injuries that result from blast exposure. Typically, lost or damaged retinal neurons in adult mammals do not proliferate or spontaneously regenerate. However, in this study, the reversal of vision loss and neurogenesis of adult retinal neurons in mice is investigated after blast exposure using a specific alpha7 nicotinic acetylcholine receptor agonist, PNU-282987, which has previously been shown to induce neurogenesis in glaucoma rodent models. In designed experiments, blast exposure is delivered to adult rodents to test the hypothesis that eye drop application of PNU-282987 reverses the loss of retinal neurons associated with blast exposure and recovers visual function. The results of these studies can lead to eye drop treatments that significantly improve visual function for soldiers that experience blast exposure in combat to improve their quality of life. In addition, the results of these studies will challenge current ideas that maintain the adult mammalian CNS is incapable of regeneration.

15. SUBJECT TERMS

NONE LISTED

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Introduction:

In designed experiments, blast exposure has been delivered to adult mice to test the hypothesis that eye drop application of PNU-282987 will reverse the loss of retinal neurons associated with blast exposure and recover visual function. These experiments are the first to determine if activation of alpha7 nicotinic acetylcholine receptors in the eye can replace neurons lost to blast exposure in adult mammals and is the first study that links alpha7 nicotinic acetylcholine receptor induced neurogenesis with change of retinal function in adult mammals.

Keywords: Blast exposure, combat trauma, neurogenesis, recovery of visual function, ERG, retina, regeneration, Muller glia, mice, transgenic, PNU-282987, alpha7 nicotinic acetylcholine receptors.

3. Accomplishments:

Two specific aims have been designed to test the hypothesis that eye drop application of the alpha7 nicotinic acetylcholine receptor agonist, PNU-282987, will reverse the loss of retinal neurons associated with blast exposure and recover visual function measured with ERG recordings.

1.To determine that eye drop application of the α7 nAChR agonist, PNU-282987, reverses the loss of retinal neurons typically associated with blast exposure.

2.To demonstrate that an increase of retinal cells after exposure to PNU-282987 affects visual function.

In order to test aim #1, 4 major tasks have been outlined. To test aim #2, two major tasks have been outlined. This is a final report. All experiments and major tasks have been completed.

Aim 1; major task 1: To establish the effect of blast exposure on neuronal survival in the retina. During the first quarter, different blast pressures on neuronal survival in the retina was assessed. During the second quarter, quantification of neuronal survival using different blast pressures was quantified. During the third quarter, we accumulated large enough "N's to complete this major task and to publish the data in "Neuroscience" under Spitsbergen et al., 2022. This information has also been presented at two different national conventions (SFN and ARVO) and at the MSHRS convention last fall in Orlando. Blast exposure using 20 psi was the minimal pressure needed to induce significant damage to retinal neurons. An average of 19% (+/- 4) of neurons were lost throughout all retinal layers using 20 psi compared to control unblasted retinas. 30 psi induced more retinal damage losing an average of 32% (+/- 8) of all neurons in all retinal layers. The maximal amount of damage that still allowed functional recovery occurred when 38 psi was used to blast the mice eyes (N=16). This resulted in a loss of 39% (+/- 8) of neuronal loss in all retinal layers. Blasts greater than 38 psi resulted in animal death a majority of the time and will not be used for the remainder of this study. The *In Press* publication resulting from this work is included as an attachment.

Major task 2 under Aim1: To examine the effect of PNU-282987 on neuronal loss after blast exposure in wild type mice and in transgenic mice containing tdTomato Muller glia. We have treated the blast exposed wild type animals to eye drops containing 1 mM PNU-282987. 1 mM PNU-282987 eye drop application was found to elicit the maximal regenerative effect in wild type mice as well as in transgenic animals. Blast exposed animals were treated with PNU-282987 for 1 week, 2 weeks and 4 weeks following the blast. At the end of these times periods, the animals were sacrificed, the retinas were removed and immunostained with antibodies for different retinal neurons in transgenic mice containing tdTomato Muller glia that allows for lineage tracing and cell counting. PNU-282987 significantly restored loss of retinal neurons due to blasts using 38 psi. This data is *In Press* for publication in Neuroscience (Spitsbergen et al., 2022) and has been presented at two national conventions as well as at MSHRS.

Major task 3 under Aim 1: To demonstrate specificity of the ACh receptor involved in PNU-282987 induced neurogenesis. When eyes were treated with 10 micromolar MLA (methyllycaconitine) before PNU-282987, the effect of PNU-282987 was significantly reduced to support the hypothesis that the PNU-282987 effect seen in figure 1B occurs through activation of alpha7nAChRs. This task is complete and the results are included in the *In Press* publication in Neuroscience (Spitsbergen et al., 2022).

Major task 4 under Aim 1: To quantify morphological changes that occur in the retina and optic nerve after PNU-282987 treatment after blast exposure. Retrograde studies using transgenic tdTomato Muller glia in adult mice treated with PNU-282987 after blast exposure have been conducted and are concluded. Neuro Vue dye paper was inserted into the optic nerve of PNU-282987 treated transgenic mice with and without blast exposure after being removed from transgenic mice. The retinas and optic nerve remained attached and were placed in 4%

PFA for several days to allow the retrograde dye to label RGC bodies in the RGC layer in wild type mice as well as in transgenic mice. In the 3rd quarterly report, images were provided to support the hypothesis that newly regenerated RGCs that originate from Muller glia extend axons into the optic nerve. For completion of this major task, measurements of cell layer thickness before and after blast exposure were obtained, quantified and compared for statistical differences. Although blast exposure significantly reduced cell numbers in all retinal layers, no statistical difference was observed in layer thickness due to the patchy nature of the blast effect. In future studies, thickness changes due to damage may be better quantified and analyzed using an OCT. The results of these changes are *In Press* in Neuroscience (Spitsbergen et al., 2022).

Aim 2; major task 1: To deliver scotopic stimulation to record rod ERG responses before and after PNU-282987 treatment in control and blast exposed adult mice. We have now completed dark-adapted ERG experiments in animals after switching from a 4 hour dark adapted period of time to a 12 hour dark adapted period of time. This longer time in the dark made a significant different during dark adapted recordings and significantly reduced the standard error between recordings. We have also completed the scoptopic ERG studies in wild type mice, after blast exposure and after treatment with PNU-282987 for 1 month after blast exposure. Blast exposure significantly decreased all wave components in the scotopic ERG responses; including the a wave, b wave, and the oscillatory potentials 1, 2 and 3. However, 1 month after eye drop treatment with PNU-282987, the ERG wave recovered significantly after the initial blast. The quantification of recovery after blast exposure under scotopic conditions has been completed and is published in Spitsbergen et al., 2022. In all instances, a blast exposure of 38 psi reduced ERG waves by approximately 50%. However, 1 month following PNU-282987 eye drop treatment, there was significant recovery of every ERG wave under every scoptopic condition.

Aim 2; major task 2: To deliver phototopic stimulation to record cone and inner retinal cell ERG responses before and after PNU-282987 treatment in control and blast exposed adult mice. Flicker responses and photopic negative responses (PhNR) ERG responses were recorded from light adapted eyes in control animals, blast exposed animals and blast exposed animals in the presence of PNU-282987. In the 3rd quarterly report, photopic ERG waveforms were recorded before and after blast exposure but low N's were obtained. Since that time, we have increased the N's of all ERG waveform recordings to between 6 and 10. When treated with PNU-282987, significant recovery of flicker responses at all frequencies and PhNR responses occurred under scotopic conditions when treated with PNU-282987 for 4 weeks. This data is *In Press* in Neuroscience (Spitsbergen et al., 2022).

Plans for next quarter: All major tasks have been completed.

4. Impact

Vision loss is responsible for reduced quality of life and a substantial burden on national healthcare systems. The results of this study could lead to eye drop treatments that significantly improve visual function in soldiers that experience blast exposure in combat to improve their quality of life. In addition, the results of this study will challenge current ideas that the adult mammalian CNS is incapable of regeneration. This proposal directly addressed functional recovery as a result of a traumatic blast event to align with one of the FY19 VRP focus areas.

No impact on other disciplines

No impact on technology transfer

No impact on society beyond science and technology

5. Changes/Problems:

This project stayed on track to complete the major tasks and milestones outlined in this award. To date, there have been no significant changes and problems encountered that prevented us from achieving the outlined milestones. Specifically, there have been no significant changes in the approach, prolonged delays,

expenditures, use of vertebrate animals, or in the use of biohazards. A minor change included the need to change the time to dark adapt mice for scotopic studies from 4 hours to 12 hours. Another minor change in the tasks included extending the number of N's from 3-5 to 5-10 for each experimental condition. Increasing the N's for each experiment reduced error bars tremendously. With these large N's, significance was typically at $P<0.01$. One other issue involved the use of the budgeted money. Due to Covid restrictions, it was difficult to bring in undergraduate students for the entirety of the project. As a result, they were limited to summer research and did not work the hours originally outlined for them. Because of this, the entire budget was not spent by the end of year two.

6. **Products:**

The following publications and abstracts have been presented based on results obtained from this study.

Peer-reviewed publication:

Spitsbergen JB, Webster SE, Linn CL. 2022. Functional changes in the adult mouse retina using an alpha7 nAChR agonist after blast exposure. *In Press Neurosci.* https://doi.org/10.1016/j.neuroscience.2022.12.107

Abstracts:

Linn DM, Spitsbergen JB, Webster SE, Linn CL. 2021. Regeneration and functional ERG recovery in a mouse glaucoma model after treatment with an alpha7 nicotinic acetylcholine receptor agonist. *SFN Abstract,* Chicago Ill.

Spitsbergen JB, Linn CL. 2021. Neurogenesis and functional recovery of adult retinal neurons in mice using an alpha7 nAChR agonist after blast exposure. *SFN abstract,* Chicago Ill.

Linn DM, Spitsbergen JB, Linn CL. 2022. An alpha7 nAChR agonist induces new RGCs and ERG recovery in a mouse glaucoma model. *ARVO abstract,* Denver CO.

Spitsbergen JB, Linn CL. 2022. Neurogenesis and functional recovery of adult retinal neurons in mice after blast exposure. *ARVO abstract,* Denver CO.

Linn CL, Spitsbergen JB. 2022. ERG functional changes associated with an alpha7 nAChR agonist after blast exposure in adult mice. *MSHRS abstract,* Orlando FL.

Linn DM, Spitsbergen JB, Webster SE, Linn CL. 2023. An alpha7 nicotinic acetylcholine receptor agonist, PNU-282987, induces new RGCs to affect pERG and pVEP recordings in a mouse glaucoma model. ARVO abstract, New Orleans LA.

7. Participants and Collaborating organizations:
No collaborating organizations are involved with this project.
Four key personnel were involved in this project including Cindy Linn (PI), Sarah Webster (graduate student), Jake Spitsbergen (graduate Student) and Hope Vanzo-Sparks (graduate student). An undergraduate student (Nastia Chan) was also recruited for these studies to perform the outlined MLA experiments to verify that PNU-282987's effects were initiated through activation of alpha7 nicotinic acetylcholine receptors.

Name:	*Cindy Linn*
Project Role:	*PI*

Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*3*
Contribution to Project:	*Dr. Linn helped to record all ERG waveforms in control untreated adult mice, as well as manage the grant requirements.*
Funding Support:	*DOD for summer research*

Name:	*Jake Spitsbergen*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*3*
Contribution to Project:	*Jake Spitsbergen set up the paintball gun to deliver blasts to adult mice eyes that leads to loss of retinal neurons. He quantified the loss of retinal cells using immunocytochemistry and confocal microscopy and recorded ERGs under scotopic and photopic conditions using the Celeris ERG system.*
Funding Support:	*DOD for summer research*

Name:	*Sarah Webster*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*3*
Contribution to Project:	*Sarah Webster performed the NeuroVue retrograde optic nerve studies to demonstrate that newly regenerated RGCs send axons down the optic nerve. She also performed immunostaining of retinal tissue using antibodies against retinal cell markers in transgenic mice.*
Funding Support:	*DOD for summer research*

Name:	*Hope Vanzo-Sparks*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*3*
Contribution to Project:	*Hope assisted with the data collecting and analysis of retinal layers as outlined in the grant proposal. In addition, she assisted with immunostaining experiments of retinal tissue using antibodies against retinal cell markers.*
Funding Support:	*DOD for summer research*

Name:	*Nastia Chan*
Project Role:	*Undergraduate Student*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*3*
Contribution to Project:	*Nastia performed the MLA experiments to verify that PNU-282987's effects were initiated through activation of alpha7 nicotinic acetylcholine receptors.*
Funding Support:	*DOD for summer research*

8. Special reporting requirements:

Quad chart for final report attached

9. **Appendices: Quad chart, Neuroscience paper**