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Hope College
141 E. 12th Sstreet
P.O. Box 9000
Holland, MI 49422 -9000

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UU	UU	UU	UU		19b. TELEPHONE NUMBER 616-395-6813

RPPR Final Report
as of 02-Mar-2020

Agency Code:

Proposal Number: 71146LSII **Agreement Number: W911NF-17-1-0559**
INVESTIGATOR(S):

Name: Gerald D Griffin
Email: griffing@hope.edu
Phone Number: 6163956813
Principal: Y

Organization: **Hope College**
Address: 141 E. 12th Sstreet, Holland, MI 494229000
Country: USA
DUNS Number: 050947084 EIN: 38-1381271

Report Date: 30-Apr-2019 Date Received: 09-Jan-2020
Final Report for Period Beginning 15-Sep-2017 and Ending 31-Jan-2019
Title: Effect of HSV-1 latent infection on laser-induced axotomy
Begin Performance Period: 15-Sep-2017 **End Performance Period:** 31-Jan-2019
Report Term: 0-Other

Submitted By: Gerald Griffin Email: griffing@hope.edu
Phone: (616) 395-6813

Distribution Statement: 1-Approved for public release; distribution is unlimited.

STEM Degrees: 3 **STEM Participants:** 7

Major Goals: The crux of the proposed and now completed work was to test the interaction between Herpes Simplex Virus Type I (HSV-1) infection and traumatic brain injury. To do this, our work tested the overall hypothesis that HSV-1 would exacerbate neuronal pathology prompted by a model of moderate brain injury. Our approach combined the use of sonic waves to disrupt neuronal structure, acute HSV-1 infection of primary neurons, and imumunodetection of the phosphorylated form of the cytoskeletal protein tau—a pathological hallmark of Alzheimer' s Disease. Our results corroborated evidence that HSV-1 increased levels of phosphorylated tau in Vero cells—a kidney epithelial cell line. Additionally, our work showed, for the first time to our knowledge, that HSV-1 infection has an additive effect with sonic wave-induced damage in primary neurons. Taken together, our work from this grant period support the notion that the existing virus in the central nervous system will lead to greater neural damage if the nervous system is disturbed by a sonic wave.

Accomplishments: Main Research Results
The crux of the proposed and now completed work was to test the interaction between Herpes Simplex Virus Type I (HSV-1) infection and traumatic brain injury. To do this, our work tested the overall hypothesis that HSV-1 would exacerbate neuronal pathology prompted by a model of moderate brain injury. Our approach combined the use of sonic waves to disrupt neuronal structure, acute HSV-1 infection of primary neurons, and imumunodetection of the phosphorylated form of the cytoskeletal protein tau—a pathological hallmark of Alzheimer's Disease. Our results corroborated evidence that HSV-1 increased levels of phosphorylated tau in Vero cells—a kidney epithelial cell line. Additionally, our work showed, for the first time to our knowledge, that HSV-1 infection has an additive effect with sonic wave-induced damage in primary neurons. Taken together, our work from this grant period support the notion that existing virus in the central nervous system will lead to greater neural damage if the nervous system is disturbed by a sonic wave.

Overall, this project supported Dr. Griffin's ability to test the interaction between HSV-1 and brain injury while training students who will advance and diversify the biomedical workforce. Three of the six students graduated in 2019—all three are working in the biomedical field—one of which is also interviewing for medical school matriculation. The other three are still undergraduate students majoring in biology or biochemistry—one of which is interviewing for entrance into dental school.

This summary report includes poster presentations as well as descriptions of results that are being prepared by undergraduates and Dr. Griffin for publication. A PDF of figures and additional accomplishments has been uploaded in the "Upload" section.

RPPR Final Report
as of 02-Mar-2020

Training Opportunities: Biomedical Workforce Training Impact
This work was executed completely at Hope College, an exclusively undergraduate institution in Holland, MI. This work involved seven undergraduate students and one high school student who worked under the direct supervision of Dr. Griffin. Of these seven Hope students, one was a freshmen student who had her first research experience due to this project. Four of the seven students were under-represented minorities. These students not only presented their work at internal research conferences at Hope but also at the annual meeting of the Society for Neuroscience (2018) as well as the Annual Biomedical Research Conference for Minority Students (ABRCMS 2018). At ABRCMS 2018, the lead presenter won the first prize in the neuroscience category; this was the first time she presented at and attended ABRCMS. One of the students was also able to utilize him time on the project to also test the direct impact of HSV-1 infection on the behavior of intact mice (results discussed later in the report). The high school student is now majoring in neuroscience at Swarthmore College.

Results Dissemination: External Presentations associated with this Award:

1. Weigle N, Da Silva C, Russell K, Griffin GD.Impact of HSV1 on an in vitro model of traumatic brain injury. 2019 Neuroscience Meeting Planner. Chicago, IL: Society for Neuroscience, 2019. Online.

2. Redding A, Weigle N, Griffin GD. Testing the impact of neuronal damage on latent Herpes Simplex Virus Type 1 infection in dorsal root ganglion neurons. Summer 2018 Biology Department Research Program. Holland, MI.

3. Da Silva C, Redding A, Weigle, N, Perez L, Griffin GD. Elucidating the impact of neuronal damage on Herpes Simplex Virus Type I (HSV-1) latency. Faculty for Undergraduate Neuroscience Social & Poster Session 2018.

4. Da Silva C, Perez L, Redding A, Weigle N, Griffin GD. Impact of neuronal damage on Herpes Simplex Virus Type I reactivation: an in vitro model. Annual Biomedical Research Conference for Minority Students 2018.

A manuscript is in preparation detailing the additive effects of neuronal damage and HSV-1 infection on phosphorylated tau levels (due to be submitted Spring 2020).

Honors and Awards: A trainee supported under this award (Clare DaSilva) won first prize in the neuroscience category for poster presentation at the Annual Biomedical Research Conference for Minority Students (ABRCMS 2018).

Protocol Activity Status:

Technology Transfer: Nothing to Report

PARTICIPANTS:

Participant Type: PD/PI
Participant: Gerald Dion Griffin
Person Months Worked: 9.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Undergraduate Student
Participant: Noah Weigle
Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

RPPR Final Report
as of 02-Mar-2020

Participant Type: Undergraduate Student
Participant: Audrey Redding
Person Months Worked: 4.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Undergraduate Student
Participant: Clare Da Silva
Person Months Worked: 3.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: High School Student
Participant: Sophie Engells
Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Undergraduate Student
Participant: Leslie Perez
Person Months Worked: 3.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Undergraduate Student
Participant: Kayla Russell
Person Months Worked: 3.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Undergraduate Student
Participant: Isaiah Hough
Person Months Worked: 4.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

RPPR Final Report
as of 02-Mar-2020

Participant Type: Undergraduate Student
Participant: Nicholai Shaw
Person Months Worked: 2.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Summary Report

Prepared by: Gerald D. Griffin, Ph.D.
Associate Provost for Academic Affairs
Associate Professor
Departments of Biology & Psychology
Hope College

Main Research Results

The crux of the proposed and now completed work was to test the interaction between Herpes Simplex Virus Type I (HSV-1) infection and traumatic brain injury. To do this, our work tested the overall hypothesis that HSV-1 would exacerbate neuronal pathology prompted by a model of moderate brain injury. Our approach combined the use of sonic waves to disrupt neuronal structure, acute HSV-1 infection of primary neurons, and imumunodetection of the phosphorylated form of the cytoskeletal protein tau—a pathological hallmark of Alzheimer's Disease. Our results corroborated evidence that HSV-1 increased levels of phosphorylated tau in Vero cells—a kidney epithelial cell line. Additionally, our work showed, for the first time to our knowledge, that HSV-1 infection has an additive effect with sonic wave-induced damage in primary neurons. Taken together, our work from this grant period support the notion that existing virus in the central nervous system will lead to greater neural damage if the nervous system is disturbed by a sonic wave.

Biomedical Workforce Training Impact

This work was executed completely at Hope College, an exclusively undergraduate institution in Holland, MI. This work involved seven undergraduate students and one high school student who worked under the direct supervision of Dr. Griffin. Of these seven Hope students, one was a freshmen student who had her first research experience due to this project. Four of the seven students were under-represented minorities. These students not only presented their work at internal research conferences at Hope but also at the annual meeting of the Society for Neuroscience (2018) as well as the Annual Biomedical Research Conference for Minority Students (ABRCMS 2018). At ABRCMS 2018, the lead presenter won the first prize in the neuroscience category; this was the first time she presented at and attended ABRCMS. One of the students was also able to utilize him time on the project to also test the direct impact of HSV-1 infection on the behavior of intact mice (results discussed later in the report). The high school student is now majoring in neuroscience at Swarthmore College.

Overall, this project supported Dr. Griffin's ability to test the interaction between HSV-1 and brain injury while training students who will advance and diversify the biomedical workforce. Three of the six students graduated in 2019—all three are working in the biomedical field—one of which is also interviewing for medical school matriculation. The other three are still undergraduate students majoring in biology or biochemistry—one of which is interviewing for entrance into dental school.

This summary report includes poster presentations as well as descriptions of results that are being prepared by undergraduates and Dr. Griffin for publication.

HSV-1 increases tau phosphorylation in Vero cells

To ensure mastery of infection and immunocytochemistry techniques and allow for corroboration of work linking HSV-1 and tau phosphorylation, we first tested the impact of HSV-1 acute infection (multiplicity of infection; MOI=1.5) on levels of tau phosphorylation in Vero cells. These kidney epithelial cells were utilized by Wozniak and colleagues (2009) where they revealed that HSV-1 increased levels of tau phosphorylation. Our results demonstrated that HSV-1 acute infection of Vero cells also prompted an increase in levels of phosphorylated tau (red). The nuclei of the cells have been stained with the fluorescent dye DAPI (blue). Qualitative analysis of the fluorescent micrographs demonstrated very intense staining outside of the nucleus of the phosphorylated form of the cytoskeletal protein tau (Figure 1). This increase of phosphorylated forms of tau has been a consistent hallmark of Alzheimer's Disease. Quantitative analysis of the immunostaining showed a statistically significant increase in mean gray values of the Vero cells expressing phosphorylated tau in the infected group (compared to uninfected cells; Figure 2). These qualitative and quantitative data support the earlier reports that HSV-1 augments of tau phosphorylation.

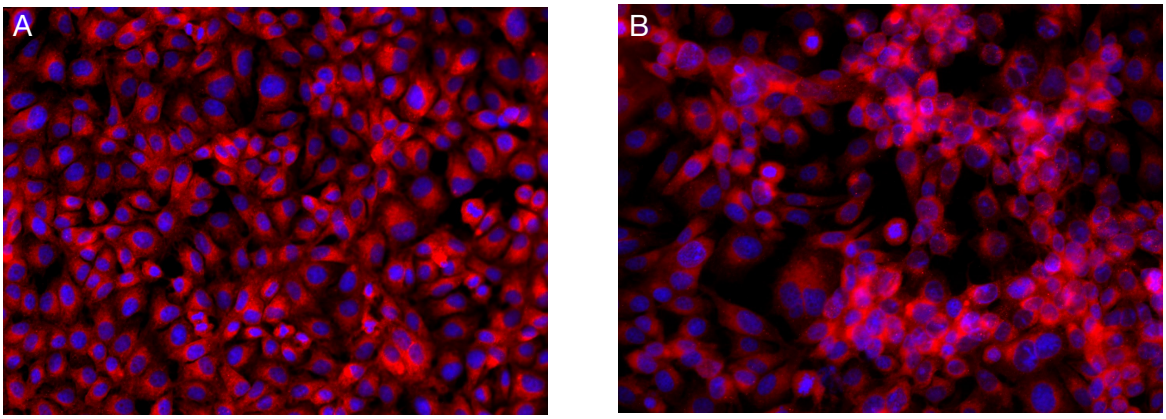

Figure 1. Immunostaining of phosphorylated tau in Vero cells is augmented by HSV-1. Vero cells were fixed with 4% paraformaldehyde before subsequent incubations with a primary antibody specific for the phosphorylated form of tau (red). All cells were treated with DAPI to localize nuclei (blue). Panel A shows cells without HSV-1 infection. Panel B shows Vero cells that have been acutely infected with HSV-1 (MOI=1.5) for 24 hours. The lower density and number cells in the infected group is indicative of the cellular loss prompted by the virus infection. There was bright staining of phosphorylated tau in the cytoplasms of cells that were infected HSV-1.

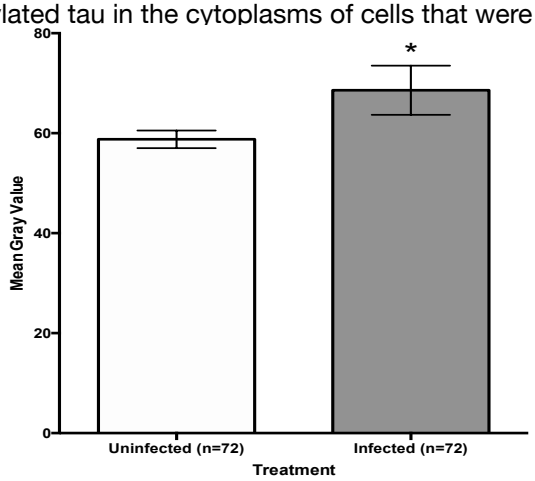

Figure 2. Acute HSV-1 infection augments immunostaining levels of the phosphorylated form of tau. The graph represents the quantitation of mean gray values of Vero cells labeled with an antibody targeted to the phosphorylated form of the cytoskeletal protein tau. Images were analyzed by ImageJ with the researcher being blind to treatment group. Vero cells infected with HSV-1 had elevated mean gray values compared to uninfected cells ($p=0.0318$, one-tailed t-test).

HSV-1 and neuronal damage augment levels of phosphorylated tau
The main goal of the proposal was to test if there was an additive effect of HSV-1 infection and neural damage, in regard to phosphorylated tau levels. To test this, we utilized rat hippocampal neuronal cultures. Undergraduates, utilizing sterile technique, plated these neurons onto L-lysine coated slides. Once adhered and stable in neurobasal media, hippocampal neurons were either: 1) left untreated (Uninfected, No Damage), 2) damaged using a sonicator for two seconds (Uninfected, Damaged), 3) infected with HSV-1 without any sanitation (Infected, No Damage), or 4) infected and subjected to sonication (Infected, Damaged). HSV-1 was infected at MOI=1.5 for 24 hours. After treatment, neurons were fixed utilized 4% paraformaldehyde and then immunolabeled to detect levels of the phosphorylated form of tau. Next, students utilized a confocal microscope to capture images of neurons. Lastly, Image J was utilized to measure the mean gray values of neurons from all four treatment groups. Qualitative analysis of images (see Figure 3) indicate intact, long neurites on neurons in the Uninfected, No Damage group (3A). Damage alone increased intensity of staining of tau phosphorylation (red) while neurites were still detectable extending from the soma (3D). Infection alone (3G) drastically reduced the presence of neurites while also enhancing staining of phosphorylated tau (compared to uninfected neurons). Slides with neurons that were both damaged and infected had much fewer cells (3J-3L) and had bright immunodetection of the phosphorylated form of tau. These qualitative results reveal that the infection did impair the integrity of neuronal structure. Moreover, HSV-1 infection and damage independently illuminated detectable levels of phosphorylated tau.

HSV-1 and neuronal damage have additive effects on phosphorylated tau levels
The quantitation of the immunolabeling of phosphorylated tau supported our main hypothesis that infection and damage interact to prompt neuronal pathology. The mean gray values of tau phosphorylation in rat hippocampal neurons were increased with damage and infection alone (compared to the Uninfected, No Damage group; $F_{3,8}=8.892$, $p=0.0063$). Additionally, the Infected, Damaged group had statistically higher levels of phosphorylated tau than the neurons that received HSV-1 but no sonication (damage). These results support the notion that HSV-1 and damage have additive impacts on neuronal pathology (Figure 4). These pieces of evidence lay the groundwork for future studies to investigate mechanisms by which HSV-1 and neuronal damage prompt the phosphorylation of tau—possibly leading to exacerbated cognitive decline in HSV-seropostive personnel subjected to traumatic brain injury.

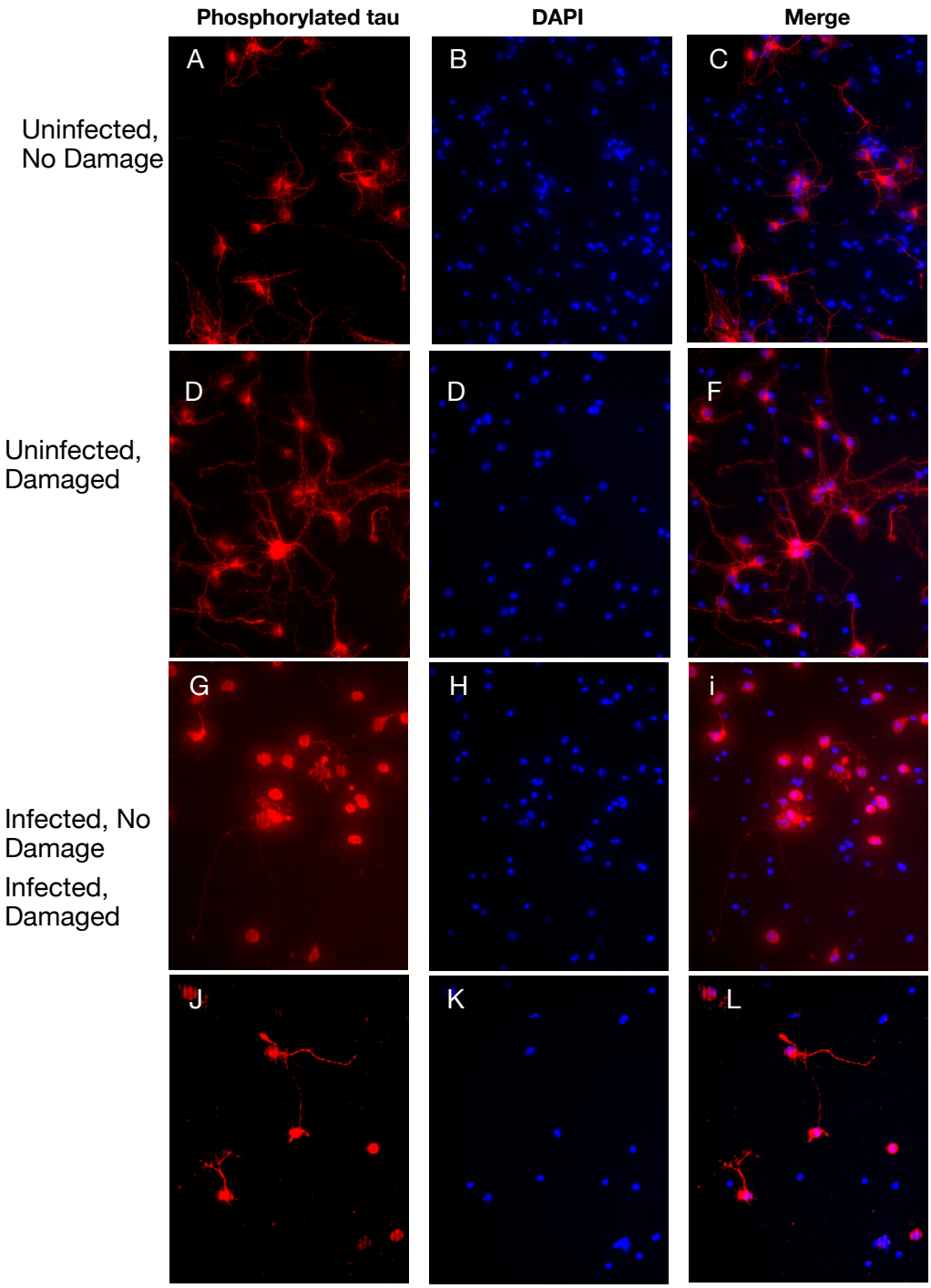

Figure 3. Independent and combined effects of neuronal damage and HSV-1 infection on tau phosphorylation in rat hippocampal neurons. Microscopy reveals staining and cellular location of phosphorylated tau (red; A, D, G, J) and the presence of nuclei via DAPI staining (blue; B, D, H, K), and merge of both signals (C, F, i, L) for neurons. Neuronal structure and basal tau phosphorylation levels are seen in the Uninfected, No Damage group. High intensity of tau phosphorylation is revealed in the Uninfected, Damaged, and Infected, No Damage groups. Additionally infection reduced the presence of neurites and condensed tau phosphorylation labeling (Infected, Damaged group).

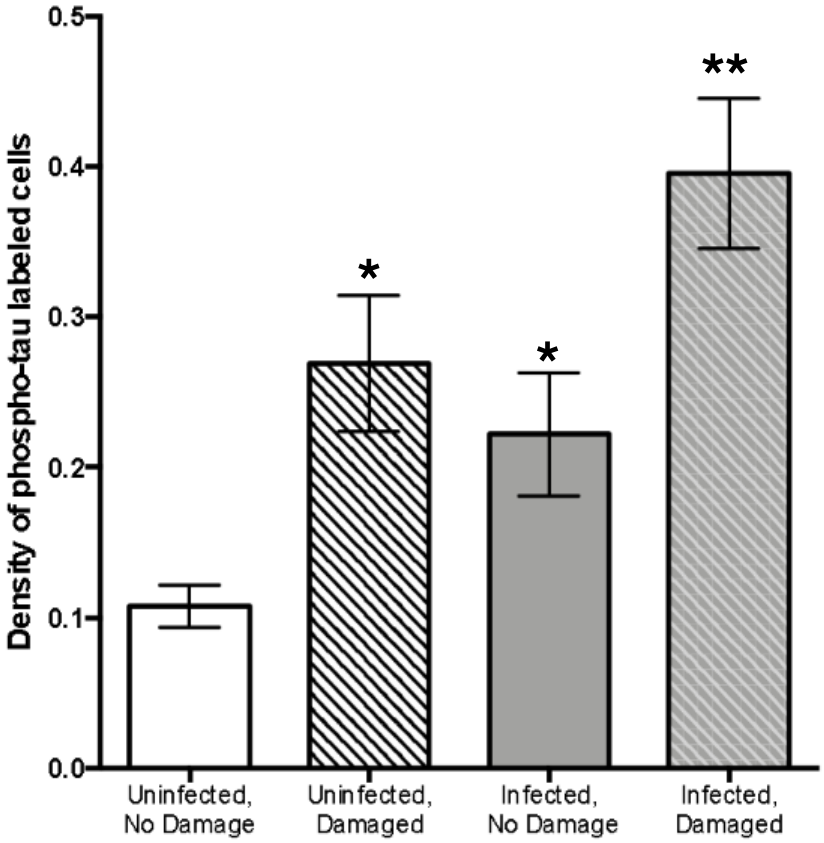

Figure 4. Infection and damage have additive effects on levels of tau phosphorylation in rat hippocampal neurons. Embryonic rat hippocampal neurons were subjected to damage via sonication, HSV-1 infection (MOI=1.5 for 24 hours), or both damage and infection. Damage alone and infection alone increased the density of cells immunopositive for phosphorylated tau (compared to the Uninfected, No Damage group; asterisk). In addition to these independent effects, the group that received both damage (sonication for 2 seconds) and HSV-1 infection had even a greater density of phosphorylated tau-labeled neurons (double asterisk) when compared to singly treated groups ($F_{3,8}$=8.892, p=0.0063).

External Presentations associated with this Award:

1. Weigle N, Da Silva C, Russell K, Griffin GD.Impact of HSV1 on an in vitro model of traumatic brain injury. 2019 Neuroscience Meeting Planner. Chicago, IL: Society for Neuroscience, 2019. Online.

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4. Da Silva C, Perez L, Redding A, Weigle N, Griffin GD. Impact of neuronal damage on Herpes Simplex Virus Type I reactivation: an in vitro model. Annual Biomedical Research Conference for Minority Students 2018.

Relation to Ongoing Project: HSV-1 increases anxiety-like behaviors
The results of this project give concrete answers to how HSV-1 impacts neuronal pathology, including its additive effect with a model of traumatic brain injury. This cellular and molecular project aligns with behavioral data our lab has accumulated showing that a latent infection of HSV-1 prompts animals (male BALB/c mice) to increase anxiety-like behavior as noted in the open field test (Figure 5A) and sociability test (Figure 5B). Analysis of other behavioral tests are ongoing. Thus far, our results demonstrated that latently infected animals—not showing evidence of viral replication—have increased anxiety-like behaviors compared to uninfected control mice.

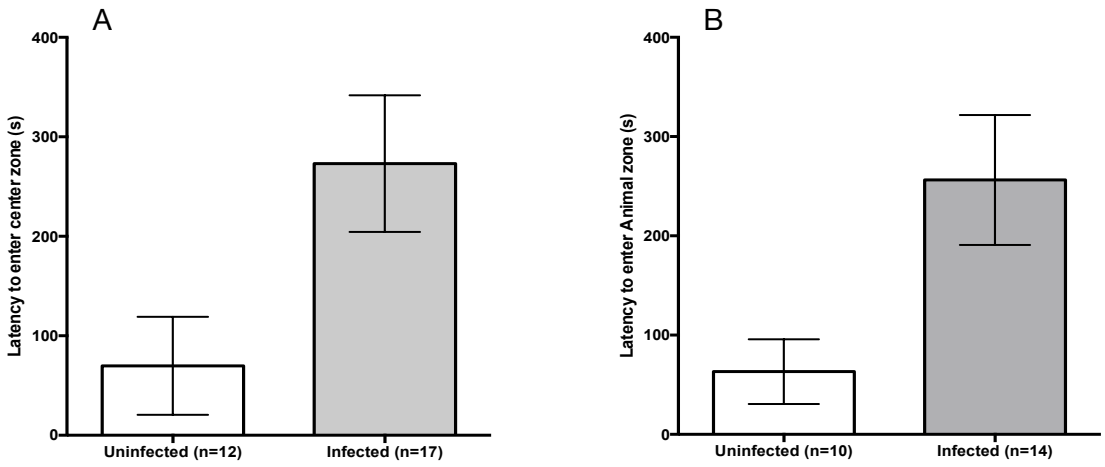

Figure 5. Latent HSV-1 infection increases anxiety-like behavior in male BALB/c mice. Panel A shows the latency (s) for male mice to enter the center zone in an open field arena. Mice were tested for ten minutes in a brightly lit open field arena. Mice infected with HSV-1 (10^6 plaque-forming units) were tested 45 days post infection, approximately two weeks after latency has begun in mice. Infected took nearly three times as long to first enter the center region compared to uninfected mice (asterisk; p=0.0178). Panel B shows the results from a sociability test where mice were free to roam in an open arena to investigate an area that either had a novel object or a novel mouse (same sex). Infected mice took longer before entering Animal zone that contained a tethered mouse compared to uninfected animals (asterisk; p=0.018).