

AWARD NUMBER: W81XWH-19-1-0606

TITLE: Neuroprotective Mechanism of DMF/MMF Associated With CAA-Related Pathology After TBI

PRINCIPAL INVESTIGATOR: Sylvain Doré PhD, FAHA

CONTRACTING ORGANIZATION: University of Florida, Gainesville, FL

REPORT DATE: September 2021

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE September 2021		2. REPORT TYPE Annual		3. DATES COVERED 15Aug2020-14Aug2021	
4. TITLE AND SUBTITLE Neuroprotective Mechanism of DMF/MMF Associated With CAA-Related Pathology After TBI.				5a. CONTRACT NUMBER W81XWH-19-1-0606	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Sylvain Doré PhD, FAHA E-Mail: sdore@ufl.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(E.S.) University of Florida Div. of Sponsored Programs 207 Grinter Hall PO Box 115500 Gainesville, FL 32611-5500				8. PERFORMING ORGANIZATION REPORT NUMBER Year 2	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(E.S.) U.S. Army Medical Research and Development Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT The effect of fumaric acid esters (DMF/MMF) on the CAA outcomes after TBI is postulated to be positive, though several discrepancies remain. Nrf2 is one of the master regulators of redox and inflammation. DMF and its metabolite MMF hold anti-oxidative and anti-inflammatory by activating Nrf2. Thus, we expect that understanding the unique and respective roles of DMF and MMF on Nrf2 on CAA neuroprotection is essential, and its validation in TBI would strengthen their potential use for the veterans and active military people. However, it is unclear whether DMF has superior beneficial effects over MMF or vice versa. Furthermore, whether the therapeutic window would differ from acute TBI over repetitive concussion-like brain insults leading to CAA needs to be tested. Considering these knowledge gaps, we aim to start answering these questions using preclinical models. For this past year, we successfully maintained/renewed the animal protocols approved by the Institutional IACUC. During the institutional COVID-19 Lab shutdown, we had to sac mice and stopped breeding; we have recently restarted breeding for the knockouts as well as the generation and characterization of the new cre-lox inducible conditional knockouts.					
15. SUBJECT TERMS None listed.					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Unclassified	18. NUMBER OF PAGES 12	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT Unclassified	b. ABSTRACT Unclassified	c. THIS PAGE Unclassified			19b. TELEPHONE NUMBER (include area code)

TABLE OF CONTENTS

	<u>Page</u>
1. Introduction	4
2. Keywords	4
3. Accomplishments	4
4. Impact	9
5. Changes/Problems	10
6. Products	10
7. Participants & Other Collaborating Organizations	10
8. Special Reporting Requirements	12
9. Appendices	12

1. Introduction

Subject: The effect of fumaric acid esters (DMF/MMF) on the CAA outcomes after TBI is postulated to be positive, though several discrepancies remain. Optimizing their respective effectiveness in both acute severe and mild repetitive head trauma is essential for the design of optimal TBI trials. Nrf2 is one of the master regulators of redox and inflammation. DMF and its metabolite MMF hold anti-oxidative and anti-inflammatory by activating Nrf2 and has been approved for multiple sclerosis and psoriasis. Thus, we expect that understanding the unique and respective roles of DMF and MMF on Nrf2 on CAA neuroprotection is essential, and its validation in two complementary TBI models would strengthen their potential use for the veterans and active military people. **Hypothesis and Purpose:** Preclinical studies raised questions, and it is unclear whether DMF has superior beneficial effects over MMF or vice versa. Furthermore, whether the therapeutic window would differ from acute TBI over repetitive concussion-like brain insults leading to CAA needs to be tested. Considering these knowledge gaps, it is essential to determine the optimal DMF and MMF therapeutic regiment and validate their respective effectiveness in two complementary TBI models. **Goals:** Aim 1 is to determine whether DMF/MMF treatment attenuates A.D. and/or CAA neurobehavioral and pathophysiological outcomes following TBI. Aim 2 is to test whether the DMF/MMF associated CAA neuroprotective mechanisms after TBI is mediated through the Nrf2 upregulation, using global Nrf2^{-/-}. Thus far, we got the animal protocols approved by the Institutional IACUC, and then by the ACURO, we have standardized the CCI, we started the breeding to generate enough global knockout mice, and finally, we also started the generation and characterization of the new cre-flox inducible conditional knockouts for the Aim 3. Though during the institutional COVID-19 Lab shutdown, we had to sac all mice and stopped the breeding, we have recently restarted the generation of knockouts and characterization of the new cre-flox inducible conditional knockouts.

2. Keywords

Alzheimer, Cerebral amyloid angiopathy, Fumaric acid esters, Transcription factor

3. Accomplishments

- What were the major goals of the project?

AIM 1

Subtask 2: Treat animals with DMF/MMF after TBI, do behavioral, harvest brains, and brain slicing

AIM 2

Major Task 2: Repeat the optimal conditions in the Nrf2^{-/-} mice

AIM 3

Major Task 3: Based on the result from Aim 2, select the first cre mice to breed with the Nrf2fl mice (and compare results with matched controls)

- What was accomplished under these goals?

AIM 1

So far, we have used 212 mice for AIM 1. CRND8 mice tend to spontaneously die, so many mice did not survive the length of the entire experiment. 160 CRND8 mice were successfully genotyped. We used 77 TgCRND8 and 83 NTgCRND8 mice. We used 50% male CRND8 mice and 50% female CRND8 mice. 181 CRND8 mice received LFAO injection, vehicle injection, or no injection. Of these mice, 149 received CCI and 100 mg/kg body weight MMF daily for 3 months. Of the mice who received CCI and MMF, 45 were survived for 3 months post-surgery, and 22 were survived for 6 months post-surgery. In total, 54 brains were harvested from CRND8 mice who underwent LFAO/control injection, CCI, and 100 mg/kg MMF treatment for 3 months. These brains will be sliced, stained with Cresyl violet, and quantified in Fall 2020. In the future, we will use more CRND8 mice to administer LFAO or vehicle injection, perform CCI, sham surgery, or no surgery, and administer methylcellulose control daily for 3 months. In the future, we will use more CRND8 mice to administer LFAO or vehicle injection, perform sham surgery or no surgery, and administer MMF or DMF daily for 3 months. Also, 16 CRND8 mice received P0/P1 10 uM LFAO ICV injections, double perfusion was performed, and their brains were harvested. Of these 16 mice, 8 were transgenic, and 8 were non-transgenic. These brains were sliced and stained with s-thioflavin and-Aβ5 immunohistostaining, and later quantified to assess the development of CAA.

Furthermore, 9 CRND8 mice received LFAO injections of different concentrations at 2 months of age. In addition, 6 of these mice received ICV injections, and 3 of the mice received hippocampal injections. Their brains were harvested 2 months later and were sliced and stained with Thioflavin-S to assess the development

of CAA. These brains will be quantified, and CAA development will be correlated with LFAO injection concentration in Fall 2020.

Subtask 2: Treat animals with DMF/MMF after TBI, do behavioral, harvest brains, and brain slicing.

We had two sample populations of WT (n=15) and Nrf2 mice (n=15), and from each population, we generated 3 groups. We were able to treat 5 mice with the vehicle, 5 mice with DMF, and 5 mice with MMF from each population before we had to sacrifice the rest of the mouse populations due to the COVID-19 institutional shutdown. This occurred before we were able to perform the behavioral studies. The brains have been harvested, and we are in the process of slicing them.

We also did CCI in male and female transgenic (TgCRND8) and non-transgenic (NTgCRND8) CRND8 mice (n~13 per group), and allowed them to age. Though because of the COVID-19 UF Lab Shutdown, we have had to sac all mice; we perfused and saved the brains we could before the institutional shutdown.

Also, 16 CRND8 mice received P0/P1 10uM LFAO ICV injections, double perfusion was performed, and their brains were harvested. Of these 16 mice, 8 were transgenic, and 8 were non-transgenic. All of these brains will be sectioned and stained with s-thioflavin and quantified to assess the development of CAA. These brains have all been sectioned via cryostat into 30 µm sections. Thioflavin-S staining was attempted, but due to these sections' thickness, we have been unable to utilize a well-functioning thioflavin-s staining method. At the time of planning the original aims, thioflavin-s seemed to be the obvious choice to analyze plaques via fluorescence. Unfortunately, we ran into the issue of utilizing thioflavin-s on 30µm sections. Comparable stains have been conducted by other labs of the UF Health Center for Translational Research in Neurodegenerative Diseases using either 5µm or 10µm paraffin sections. Therefore, we attempted to develop a standardized staining procedure for our 30µm frozen sections. For our first attempt, we started a standardized protocol utilized by Dr. Yona Levites, subtracting any deparaffinized tissue steps. This process followed the standard thioflavin-s protocol of ethanol incubation, thioflavin-s incubation then washing in ethanol and PBS. Additionally, this standardized procedure included an autofluorescence eliminator reagent; however, this solution stuck too much to the 30µm sections and blocked out any successful fluorescence. Dr. Levites recommended we consult Dr. Guilian Xu, also of the UF Health CTRND. Dr. Xu utilizes a standardized Guntern Modification of classic Thioflavin-S staining modified from the Johns Hopkins University Department of Neuropathology. She conducted the stain as she has in the past with, unfortunately, no success in visualizing fluorescent plaques. We again attempted Thioflavin-S staining on 08/26/21, using Sudan Black B in place of an autofluorescence eliminator reagent. The attempt succeeded in having some fluorescence, though the fluorescence was not as distinct as we expected, especially in the TgCRND8 mice. After some troubleshooting, we decided to forgo the use of our normal Permout mounting medium, as it is not ideal for fluorescent stains. Instead, we plan to use 90% glycerol: PBS to mount our slides, sealing them with nail varnish. Now that we have a working thioflavin-s protocol, we hope to use a Zeiss Axioscan.Z1 to scan these slides, though we are waiting for training from Zeiss since our lab has only used a Leica Aperio AT2 scanner in the past. The latter only offers brightfield microscopy, and we need the fluorescent microscopy that the former can offer.

While we were optimizing the thioflavin-s protocol, we sought the help of the UF Health CTRND Histology Core and the lab of Dr. Todd Golde, also of the UF Health CTRND. The decision was reached that we should have a contingency should the Thioflavin-S protocol continues to fail. Therefore, we planned to utilize a standardized protocol from the UF Health CTRND Histology Core and Ab5 antibodies provided by Dr. Todd Golde's lab to conduct a 3,3'-Diaminobenzidine (DAB) antibody stain on these sections to visualize amyloid-beta plaques. This stain is similar to other immunohistochemical stains we have conducted in the past, so we hope it can serve as an effective backup. We have successfully performed anti-Ab5 staining on slides from the 16 LFAO treated brains, and we are currently working to scan and quantify them using Aperio ImageScope.

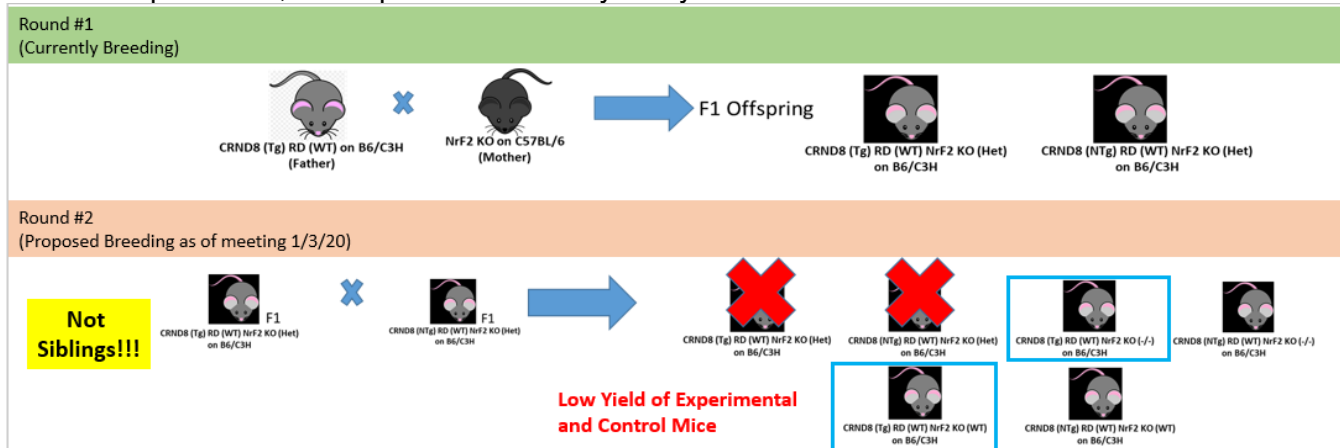
So far, we have performed Cresyl violet, Perls Prussian Blue, and Luxol Fast Blue stains, as well as Iba-1, GFAP, and Ab5 IHC stains on the 16 LFAO treated brains. Unfortunately, some damage was done to the brain sections during this unusually long storage (because of COVID-19), but the stains still provided useful data. We have scanned the Cresyl violet, Iba-1, and Luxol Fast Blue stains, and we are currently in the process of quantifying and analyzing the data.

AIM 2

Major Task 2: Repeat the optimal conditions in the Nrf2^{-/-} mice

We had to sacrifice the CRND8 x Nrf2^{-/-} colony due to poor breeding outcomes. However, the brains from these animals have been harvested and will be used by staff to practice slicing before slicing the experimental

brains from AIM 1 to ensure that the experimental brains will be sliced properly and can be stained and quantified. We discovered that we would have better breeding outcomes in the F1 generation if the CRND8 gene came from the male mouse. We restarted the CRND8 x Nrf2^{-/-} colony using 5 CRND8 male and 5 Nrf2^{-/-} female mice. We will recreate the F1 generation using better breeding methods, genotype the mice, use these mice for experiments, and replenish the colony every 6 months or as needed.



As a plan for this, we have also already started the proper breeding strategy and colonies to have the mice needed.

We generated from test/preliminary data to look at the W.T. and Nrf2^{-/-} outcomes following CCI. We are pleased to report that the Nrf2^{-/-} mice survive following the TBI, suggesting that our CCI protocol is ready for use with experimental mice. We analyzed the data for significance using one-way ANOVA.

From the Nrf2^{-/-} population, we treated 5 mice with a vehicle, 5 mice with DMF, and 5 mice with MMF. Since slicing with previously done by undergraduate volunteers, this has been slowed down because of COVID19 and the complete turnover of our volunteer roster; it will resume now that students are returning and are training in the methodology involved in frozen sectioning. Some preliminary behavioral data was generated from the mice treated with vehicle, DMF, and MMF. While no significant differences were found at 24h, at 72h the WT TBI+vehicle group had significantly higher NDS compared to WT TBI+DMF (ANOVA: $p < 0.01$) and WT TBI+MMF (ANOVA: $p < 0.01$). Rotarod test results corroborated these results, as there were no significant differences at baseline of 24h. Still, the WT TBI+vehicle group had significantly lower latency of fall compared to WT TBI+DMF (ANOVA: $p < 0.01$) and MMF (ANOVA: $p < 0.01$) groups. There were no significant differences between DMF- and MMF-treated groups. These results were reversed in the Nrf2^{-/-} group, suggesting that any neuroprotective effect of DMF/MMF is mediated by Nrf2. We hope to investigate further these results in TgCRND8 mice and TgCRND8+Nrf2^{-/-} mice (Fig. 1).

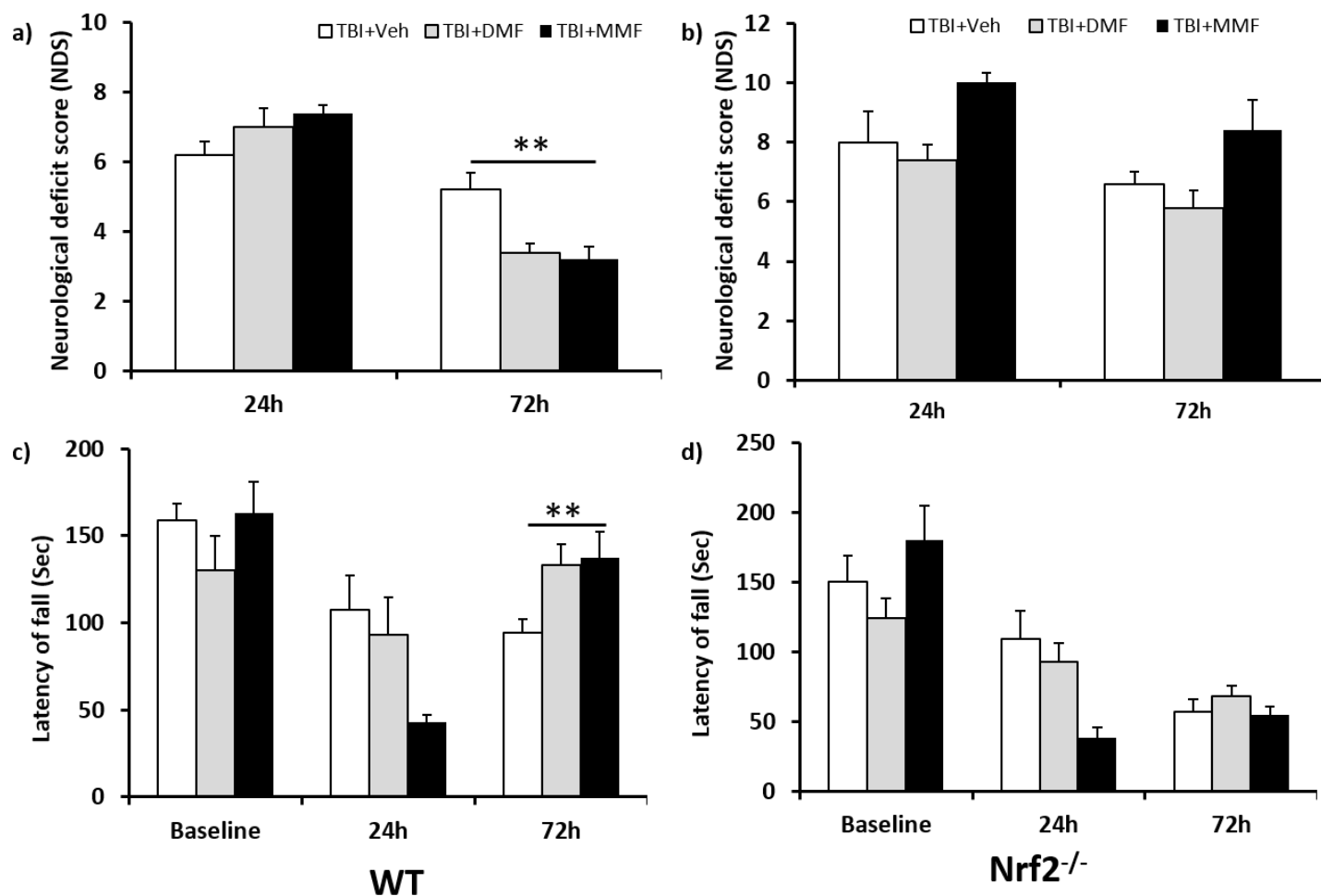


Figure 1. Neurological deficit score (NDS) at 24h and 72h for (a) WT and (b) *Nrf2*^{-/-} mice treated with either DMF, MMF, or a vehicle. Results of rotarod test measured in latency of fall (sec) at 24h and 72h for (c) WT and (d) *Nrf2*^{-/-}. *= $p < 0.05$, ** $p < 0.01$

In addition to performing NDS assessments and rotarod tests, we also performed open field tests. Unlike the previous functional assessments, significant differences were found at 24h for the open field test for both TBI+DMF and TBI+MMF in terms of ambulatory distance ($p < 0.01$ vs. TBI+Veh) and stereotypic count ($p < 0.05$ vs. TBI+Veh). Furthermore, these significant differences persisted at 72h for both TBI+MMF ($p < 0.01$ vs TBI+Veh).

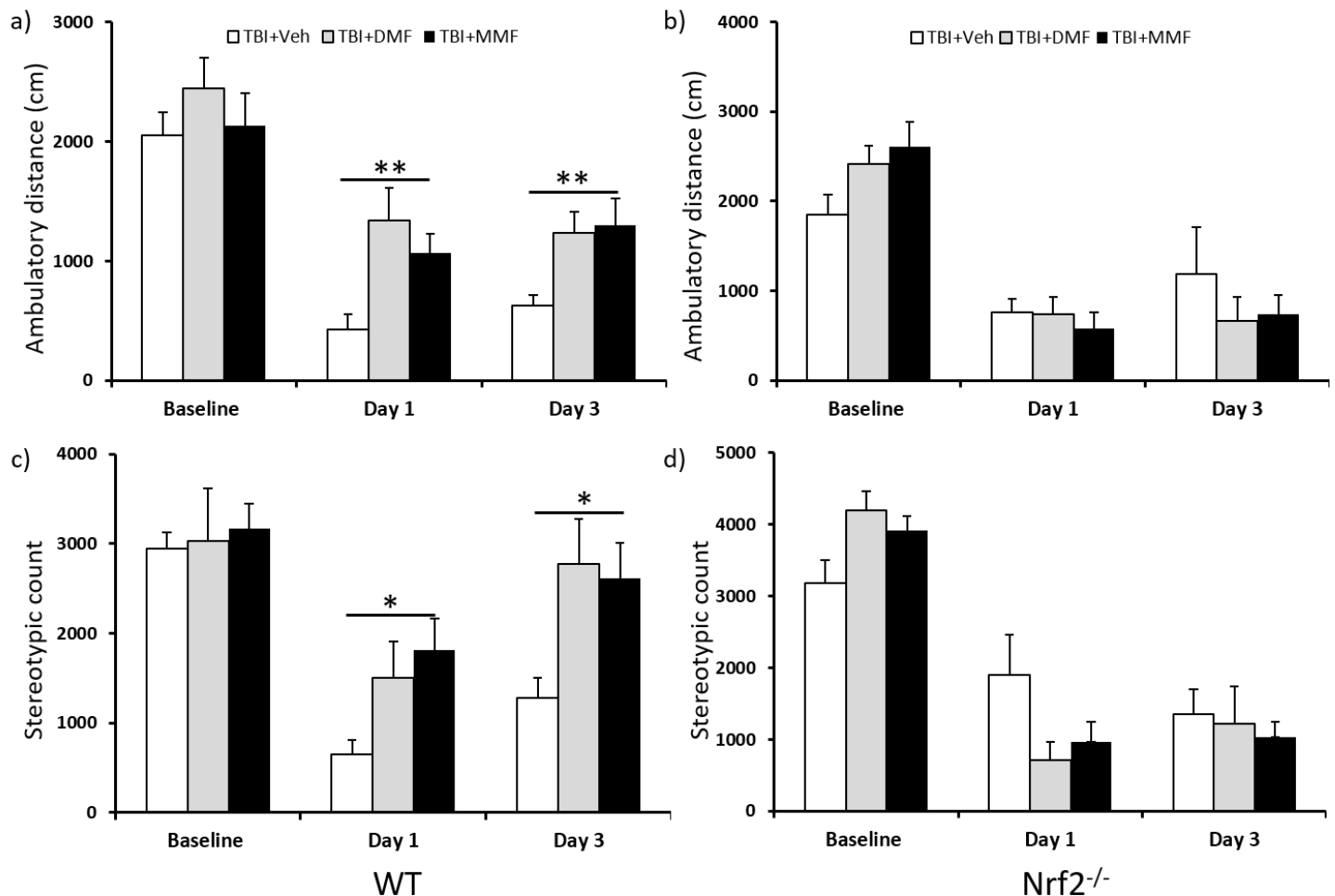


Figure 2. Results of the open field distance measured in the ambulatory distance (cm) at 24h and 72h for (a) WT and (b) Nrf2^{-/-} mice treated with either DMF, MMF, or a vehicle. Results of passive avoidance test measured in the stereotypic count at 24h and 72h for (c) WT and (d) Nrf2^{-/-}. *p<0.05, **p<0.01.

The brains have been harvested, and we are in the process of slicing them. In addition, we have recruited 1 technician who was previously in the lab and had experience with sectioning to train our undergraduate volunteers and future technicians.

AIM 3

Major Task 3: Based on the result from Aim 2, select the first cre mice to breed with the Nrf2^{fl} mice (and compare results with matched controls)

As we plan for this, we are actively continuing the breeding of Nrf2^{flox} mice with the various cre mice. We started with the cre neuronal (Cx3-Cre), cre astrocytic (GCE), and cre microglial (CCE).

As these are inducible knockouts (which reduced compensatory mechanisms as compared to neonates cre-flox). We have now set up the optimal protocols for the tamoxifen treatment to induce the recombination.

We also started the characterization for the efficiency of the recombination (this is mostly done by PCR; we have now established standard protocols).

We are pursuing the breeding strategy as planned, and the F1 generation has already been crossed and weaned. Genotyping has been completed for all weaned mice, and the experimental mice are being aged until ready for use with experimental protocols.

- What opportunities for training and professional development has the project provided?

Nothing to Report.

- How were the results disseminated to communities of interest?

We have listed the DOD grant as a source of funding for 5 accepted publications and 3 submitted publications:

Ashouri R, Fangman M, Brielmaier J, Fields ZA, Campo N, **Doré S**. Nutritional Supplementation of Naturally Occurring Vitamin D to Improve Hemorrhagic Stroke Outcomes. *Front Neurol*. 2021; 12: 670245.

Cavicchioli F, Cesarotti IM, Fangman M, Lua J, Hautamaki R, **Doré S**. Carbon Monoxide Therapy Using Hybrid Carbon Monoxide-Releasing/Nrf2-Inducing Molecules through a Neuroprotective Lens. *Chemistry*. In Press 07/23/2021

Ashouri R, Fangman M, Burris A, Ezenwa MO, Wilkie DJ, **Doré S**. Critical Role of Hemopexin Mediated Cytoprotection in the Pathophysiology of Sick Cell Disease. *Intl J Mol Sci*. 2021 Jun 15;22(12):6408. Doi:10.3390/ijms22126408. PMID: 34203861, PMCID: PMC8232622.

Fu K, Garvan CS, Heaton SC, Nagaraja N, **Doré S**. Association of Serum Bilirubin with the Severity and Outcomes of Intracerebral Hemorrhages. *Antioxidants* 2021, 10, 1346

Lua J, Ekanayake K, Fangman M, **Doré S**. Potential Role of Soluble Toll-Like Receptors 2 and 4 as Therapeutic Agents in Stroke and Brain Hemorrhage. *Intl J Molec Sci*, In Press 09/12/2021

Mazur A, Fangman M, Ashouri R, Pamplin H, Patel S, **Doré S**. Carbon Monoxide in Acute Brain Injury and Neuroprotection. Carbon Monoxide in Drug Discovery: Basics, Pharmacology, and Therapeutic Potential. Submitted 02/5/2021

Lichlyter DA, Krumm ZA, Golde TA, **Doré S**. Stress response to stroke and support for CRF antagonist treatment. *FEBS Journal*. Submitted 06/1/2021

Fangman M, Ashouri R, Golovachev N, **Doré S**. Role of the brain-permeable HBED iron chelator in preserving gray and white matter and overall reducing oxidative stress after TBI. *Neurochem. Intl*. Submitted 08/26/2021

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

AIM 1

Subtask 2: Treat animals with DMF/MMF after TBI, do behavioral, harvest brains, and brain slicing

Despite the loss of most of our trained volunteers, we have been slowly building back up a base of undergraduate volunteers who can assist us in tasks such as brain slicing. The DMF/MMF treated brains have already been harvested. Although we were never able to fully complete the behavioral testing before the institutional shutdown, there are still preliminary behavioral data generated from WT and Nrf2^{-/-} mice (and none from the TgCRND8 mice). Currently, our undergraduate volunteers have been trained to section brains and have completed the LFAO treated brains. They are now moving on to the DMF/MMF treated TBI surgery brains.

Subtask 3: Analyze lesion volume and behavioral

None of the LFAO treated brains had CCI done before they were sacrificed. Therefore, we are currently sectioning the MMF/DMF treated brains, and we will perform Cresyl violet stains to analyze lesion volume.

Subtask 4: Perform various stainings/assays, do quantifications, and complete analyses

Subtask 5: Monitor toxicity at the different doses

Once we have an adequate number of experimental and control mice, we hope to administer different doses and track toxicity and organ damage markers via blood samples and brain sectioning.

AIM 2

Major Task 2: Repeat the optimal conditions in the Nrf2^{-/-} mice

Continue the breeding strategy to have the mice needed. We have now recruited 1 tech to restart fully.

AIM 3

Major Task 3: Based on the result from Aim 2, select the first cre mice to breed with the Nrf2^{fl} mice (and compare results with matched controls)

Continue to breed and complete the characterization of these unique cre-flox mice. Such that once needed, then we have the mice ready for the planned protocol. Though because of the COVID-19 UF Lab Shutdown, we had to sac many animals. We have now recruited 1 tech to restart entirely.

4. Impact

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

Nothing to Report.

- What was the impact on other disciplines?

Nothing to Report.

- What was the impact on technology transfer?

Nothing to Report.

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

Nothing to Report.

5. Changes/Problems

Nothing to Report.

6. Products

Nothing to Report.

7. Participants & Other Collaborating Organizations

- What individuals have worked on the project?

Name:	<i>Sylvain Doré, PhD, FAHA</i>
Project Role:	<i>PI</i>
Researcher Identifier (e.g. ORCID ID):	<i>0000-0003-3771-5109</i>
Nearest person month worked:	<i>2</i>
Contribution to Project:	<i>S.D. has been managing the project and coordinated the breeding, etc.</i>
Funding Support:	<i>NIH-NINDS, NINR</i>

Name:	<i>Yona Levites, PhD</i>
Project Role:	<i>Co-I</i>
Researcher Identifier (e.g. ORCID ID):	<i>0000-0001-6925-4525</i>
Nearest person month worked:	<i>1</i>
Contribution to Project:	<i>Y.L. is assisting with the mouse injections and the use of the CRND8 mice.</i>
Funding Support:	<i>N/A</i>

Name:	<i>Abdullah Shafique Ahmad, PhD</i>
Project Role:	<i>Ph.D. Scientist</i>
Researcher Identifier (e.g. ORCID ID):	<i>0000-0002-8368-2443</i>
Nearest person month worked:	<i>1</i>
Contribution to Project:	<i>A.S.A. has been assisting S.D. in TBI protocols. He was recruited for promotion by another institution as of Sept. 7th, 2020.</i>
Funding Support:	<i>N/A</i>

Name:	<i>Kristy Dillon</i>
Project Role:	<i>Biological Scientist</i>
Researcher Identifier (e.g. ORCID ID):	<i>N/A</i>
Nearest person month worked:	<i>2</i>
Contribution to Project:	<i>K.D. is providing tech and breeding support for the mice</i>
Funding Support:	<i>N/A</i>

Name:	<i>Alexandra Margaret Mazur</i>
Project Role:	<i>Lab Technician</i>
Researcher Identifier	<i>0000-0002-4280-9065</i>

(e.g. ORCID ID):	
Nearest person month worked:	3
Contribution to Project:	<i>A.M.M. is providing lab support for the various protocols. She has left for medical school as of January 29th, 2021.</i>
Funding Support:	NIH-NINDS

Name:	<i>Josh Antonio Lua</i>
Project Role:	<i>Lab Technician</i>
Researcher Identifier (e.g. ORCID ID):	0000-0003-0749-3314
Nearest person month worked:	2
Contribution to Project:	<i>J.L. is providing lab support for all current protocols</i>
Funding Support:	N/A

Name:	<i>Raymond Cole Hautamaki</i>
Project Role:	<i>Lab Technician</i>
Researcher Identifier (e.g. ORCID ID):	0000-0002-0758-7495
Nearest person month worked:	2
Contribution to Project:	<i>R.C.H. is providing lab support for the various protocols. He has left due to family matters as of June 30th, 2021.</i>
Funding Support:	N/A

Name:	<i>Rani Farid Ashouri</i>
Project Role:	<i>Lab Technician</i>
Researcher Identifier (e.g. ORCID ID):	0000-0003-2423-6723
Nearest person month worked:	5
Contribution to Project:	<i>R.A. is providing lab support for the various protocols. He has left for medical school as of May 12th, 2021</i>
Funding Support:	NIH-NINDS

Name:	<i>Madison Michelle Fangman</i>
Project Role:	<i>Lab Technician</i>
Researcher Identifier (e.g. ORCID ID):	0000-0002-0092-1956
Nearest person month worked:	9
Contribution to Project:	<i>M.F. is providing lab support for the various protocols. She has left for future endeavors as of May 20th, 2021</i>
Funding Support:	N/A

Name:	<i>Andrew Daniel Lichlyter</i>
Project Role:	<i>Lab Technician</i>
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	4
Contribution to Project:	<i>D.L. is providing lab support for all current protocols</i>
Funding Support:	N/A

- Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

ASA was recruited by another institution as of Sept. 7th, 2020. We are now posting a Senior Level Position, and have identified a senior postdoc with appropriate experience. We have also been recruiting 1 other additional full-time technician to assist in catching up with the proposed aims; the tech was previously trained in the lab with more than 1.5-2yrs of experience.

- What other organizations were involved as partners?

Nothing to Report.

8. Special Reporting Requirements

Nothing to Report.

9. Appendices

Quadchart