Award Number: W81XWH-10-1-0774

TITLE: Theory-Driven Models for Correcting “Fight or Flight” Imbalance in Gulf War Illness

PRINCIPAL INVESTIGATOR: Gordon Broderick, Ph.D.

CONTRACTING ORGANIZATION: University of Alberta
Edmonton, Alberta, Canada, T6G 2C8

REPORT DATE: September 2011

TYPE OF REPORT: Revised Annual

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

DISTRIBUTION STATEMENT:
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<td>University of Alberta Dept. of Medicine Suite 225B College Plaza, 8215 112 Street NW, Edmonton, AB, Canada T6G 2C8</td>
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<td>The objective of this study is to create a comprehensive engineering model of endocrine-immune interaction dynamics in order to identify (i) theoretical failure modes of the HPA-immune axis that align with GWI, and (ii) promising treatment strategies that exploit the regulatory dynamics of these systems to reset control of the HPA-immune axis to normal.</td>
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We have completed the negotiation of sub-awards to the CFIDS Association of America, the university of Miami and Ohio State University. The ratification of these inter-institution agreements required more time than originally anticipated and on August 29, 2011 we requested a one-year extension without additional funding to adjust for this delay. Some limited preliminary work was nonetheless conducted. We are recruiting a new senior research associate and expect no further delays.

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**Introduction**

The hypothalamic-pituitary-adrenal (HPA) axis controls the body’s “fight or flight” response through a series of endocrine and immune signals directed at ensuring immediate survival and later re-establishing homeostasis. Changes in the tone of this response have been observed in veterans with Gulf War Illness (GWI). Studies report abnormal cell proliferation, impaired function and persistent oxidative stress in circulating immune cells of patients. Similarly dysregulation of the HPA axis includes hypersensitivity in cytokine feedback as well as suppression cortisol and neurotransmitters responsible for mediating innate and adaptive immunity.

We propose that severe physical or psychological insult to the endocrine and immune systems can displace these from a normal regulatory equilibrium into a compromised stable state. This state is characterized by a self-perpetuating inflammatory response that is left uncorrected due to deficient HPA axis control. To explore the validity of this hypothesis we will create a comprehensive engineering model of endocrine-immune interaction dynamics in order to identify (i) theoretical failure modes of the HPA-immune axis that align with GWI, and (ii) promising treatment strategies that exploit the regulatory dynamics of these systems to reset control of the HPA-immune axis to normal.

**Body.**

A fully executed copy of the basic award was received August 23, 2010 and the project start date was set as September 1, 2010. The corresponding institutional PIF form was accepted by the DOD payment office October 7 and the main account was established December 9, 2010. Concurrently the University of Alberta initiated the sub-grant agreement process the first week of November with all 3 sub-grantees: the CFIDS Association of America, the University of Miami and Ohio State University. These sub-award agreements were only ratified by each of the 3 collaborating institutions in late May 2011. As a result of this significant delay the University of Alberta’s Research services Office submitted on behalf of the principal investigator a request for a one-year extension of the project term September 7, 2011 (Appendix A). This request was reviewed by Ms. Strock of the DoD and we were asked to resubmit this request at a later date (6-8 months before end of project term). We have since confirmed with Ms. Strock that this continues to be the correct course of action (ref. email from Dr. Phillips, Ms. Strock, dated January 23, 2012; Appendix A).

During this time a personnel search was initiated for a senior research associate. Work was conducted on a limited basis by an interim research associate under the auspice of a fixed-term contract that terminated on September 1, 2011. Activities of this interim research associate focused mainly on the recovery and reassessment of the basic model reported in Ben-Zvi et al., 2009 [1]. This work is consistent with Task 3 in the original Statement of Work (SOW) and is described in the following section.

**A preliminary analysis of circadian rhythm in fatiguing illness clinically relevant to GWI**.

Our earlier work [1] demonstrated the existence of multiple steady states in the HPA axis. This work did not formally consider the 24-hour cycle of cortisol expression. To explore the importance of this effect we obtained clinical data describing blood levels of adrenocorticotropic hormone (ACTH) and cortisol taken over a 24-hour period at 10-minute intervals. Detailed descriptions of the patient cohort and data collection methods are available in Crofford, et al. [2]. Female subjects were clinically assessed for chronic fatigue syndrome (CFS), fibromyalgia (FM) or both (combined CFS/FM). Each test subject was matched to a healthy control subject. Two subjects were removed from the data set due to an excessive number of missing values, leaving 14 subjects with CFS, 10 subjects with FM, and 11 subjects with combined CFS/FM, for a total of 35 patients. The dataset also contained corresponding information for 35 matched, healthy control subjects. In this exploratory analysis, we focused principally on data from subjects with a single diagnosis, either CFS or FM, and their corresponding control subjects.

A typical cortisol time course in a healthy test subject is shown in Figure 1 and a typical ACTH time course is shown in Figure 2. Figures 1 and 2 show both the data and values predicted by a modified cosinor model based on an expected 24-hour period (Eq. 1). In this model we simultaneously fitted a linear trend, a sine wave with a 24-hour period and a sine wave of the first higher harmonic of the 24-hour period. These components represent the low frequency content of each signal, including slow linear trends and drift. Because of the identity \( \sin(\omega t + \phi) = \cos(\phi) \sin(\omega t) + \sin(\phi) \cos(\omega t) \), simultaneous fitting of both sine and
cosine terms of the same frequency is mathematically equivalent to fitting a sine wave containing a phase shift.

\[ \hat{y} = a_0 + a_1t + b_1 \sin(\omega t) + b_2 \cos(\omega t) + c_1 \sin(2\omega t) + c_2 \cos(2\omega t) \]  

Eq. 1

The trends for cortisol and ACTH values averaged point wise across all healthy subjects are shown in Figures 3 and 4. A very strong 2-hour periodic tendency was observed. This signal could not be easily explained as an artifact of the data collection procedures and high-frequency burst patterns in cortisol have been reported [3]. As a result this 2-hour component in the average cortisol time course was investigated further using a more detailed frequency domain analysis based on the discrete Fourier transform (DFT). We applied the DFT using Matlab’s fft command. In computing Fourier coefficients, we de-trended the time series by subtracting the low frequency terms in Eq. 1; therefore, the Fourier coefficients corresponding to frequencies 0, 2\( \pi \)/24 and \( \pi \)/24 are forced to zero. We viewed this as appropriate because our analysis focused on higher frequency content of the signals because the existence of low-frequency circadian rhythms in cortisol and ACTH are well known. Results shown in Figure 5 describe the ratio of power of the signal at a specific frequency to the overall background power as described in work by Shumway [4]. These suggest that in CFS the fluctuations in cortisol occurring on a 2-hour (0.5 hr\(^{-1}\)) and 40-minute (1.5 hr\(^{-1}\)) period differ significantly from background (red dotted line) and are may be suppressed in the illness group. In the case of ACTH fluctuations, only the 40-minute period is suppressed (Figure 6). Though these results were obtained in a female population of CFS patients, a significant overlap in clinical presentation between CFS and GWI has been reported [5, 6]. As a result we would like to pursue this line of investigation with a possible analysis of 24-hour ACTH and cortisol levels measured in GWI patients by Dr. Julia Golier at the Bronx VA Medical Center. We hope to contact Dr. Golier in the first quarter of 2012 to discuss this possibility. The presence of potential illness-related discrepancies in cortisol fluctuations at these frequencies suggests that the dynamics for a reservoir of bound cortisol should be considered for inclusion into the model.

**Key Research Accomplishments.**

In keeping with the milestones described in the project submission initial efforts were directed at:

- We recovered the details of the model published in 2009 and re-encoded these into the MatLab environment: a high-level programming and simulation software. This was integrated with functions available under TomLab, a nonlinear optimization package designed for use with MatLab. Together these components support the tuning of model parameters to experimental data as well as the calculation of optimal treatment scenarios.
- We conducted some initial parameter tuning and sensitivity analysis based on measurements of the hormones ACTH and cortisol measured under a sister project at 3 points during the course of an exercise challenge. This work is ongoing.
- We assessed the natural frequencies supported by the basic model and found the latter did not naturally support circadian rhythm without an external forcing function.
- We conducted an expanded analysis of circadian rhythm in a separate data set describing circadian rhythm in ACTH and cortisol in female patients with a closely related illness, chronic fatigue syndrome (CFS) [2]. Results indicated that low-frequency circadian cycles might not be significantly altered in these patients compared to match controls. However differences might exist at higher frequencies and burst emissions of cortisol should be considered for inclusion into the model possibly as a compartment for bound cortisol. Such a compartment was absent from the initial model even though bound cortisol was identified as a therapeutic target.
- These initial trials on limited data also suggested that a linear model of the adrenal gland might be limited in terms of fidelity. A non-linear model should be considered.
- Initiated literature search for detailed mathematical models of the hypothalamic-pituitary-adrenal axis that account for high-frequency changes in free cortisol. These included but were not limited to work by Veldhuis and colleagues [3].
**Reportable Outcomes.**
The results of a preliminary analysis describing the frequency decomposition circadian shifts in blood cortisol were presented in a roundtable discussion with the University of Miami team in Miami the week of March 21, 2011.

**Conclusions.**
We have requested an extension of the project term due to a delayed start. This request is currently being reviewed by the DoD. A project-specific research associated is currently being recruited to commence activities in earnest. Agreements have now been ratified with all 3 collaborating institutions and we expect no further delays.

**References.**
Figure 1. Typical 24-hr cortisol time course in a healthy control subject from the data of Crofford et al. [2].

Figure 2. Typical 24-hr ACTH time course in a healthy control subject in data from Crofford et al. [2].
Figure 3. 24-hr Cortisol time course for the point-wise mean of all healthy control subjects reported in Crofford et al. [2].

Figure 4. 24-hr ACTH time course for the point-wise mean of all healthy control subjects in the same cohort described in Figure 3.
Figure 5. Frequency decomposition of 24-hr cortisol time course for CFS patients and matched control subjects reported in Crofford et al. [2]. The power at each frequency is expressed as a ratio of signal to noise. The threshold (red dotted line) above which a frequency is estimated to have a non-zero representation is based on the modified Hartley statistic [4].

Figure 6. Frequency decomposition of 24-hr ACTH time course for CFS patients and matched control subjects. The power at each frequency is expressed as a ratio of signal to noise as in Figure 5.
Appendix A: Request for Extension

Research Services Office
222 Campus Tower 825 - 112 Street NW
Edmonton, Alberta, Canada T6G 2E1

August 29, 2011

Abigail Diffenderfer
Contract Specialist, MCMR-AAA-E
U.S. Army Medical Research Acquisition Activity
820 Chandler Street
Fort Detrick, MD 21702-5014
USA

Dear Ms. Diffenderfer:

RE: W81XWH-10-1-0774
University of Alberta File: 0001076 (Dr. Gordon Broderick)

It is in regards to the above mentioned contract and on behalf of the Principal Investigator, Dr. Gordon Broderick that I write you this note. We would be grateful if a one (1) year no-cost extension from the US Department of Defence could be granted for the above noted research grant. This request is due to circumstances beyond the control of Dr. Broderick but rather, the time it took to finalize the inter-institutions sub-grants. These subgrants have only recently been signed-off which puts Dr. Broderick’s research a year behind schedule. As such it would be most helpful to Dr. Broderick and the sub-sites if the end date of the project could be moved from August 31, 2013 to August 31, 2014.

Sincerely,

Juliane Herst
Assistant Director - Health Sciences

c: Dr. Gordon Broderick

Research Services

www.ro.ua/uberta.ca info@ro.ua/uberta.ca
Tel: 780.492.5781 Fax: 780.492.5961

RESEARCH SERVICES
10
Excerpt from email correspondence with Dr. Phillips and Ms. Strock regarding submission of request for extension.

From: "Phillips, James B Dr DoD A f US USA MEDCOM CDMRP" <James.Phillips19@us.army.mil>
Subject: R E : Report Disapproved - DOD Award (10-1-0774) Review of ANNUAL REPORT GW093042 (UNCLASSIFIED)
Date: January 25, 2012 10:06:45 AM MST (CA)
To: "Strock, Abigail I Ms CIV USA MEDCOM USAMRAA" <ABIGAIL.L.STROCK@US.ARMY.MIL>, Gordon Broderick <gordon.broderick@ualberta.ca>
Cc: "Fisher, Rebecca A Dr CTR US USA MEDCOM CDMRP" <Rebecca.Fisher1@us.army.mil>

Classification: UNCLASSIFIED
Caveats: NONE

Thanks Abby!

I'd suggest between 6-8 months of the end date of the award.

JB

-----Original Message-----
From: Strock, Abigail I Ms CIV USA MEDCOM USAMRAA
Sent: Monday, January 23, 2012 8:01 AM
To: Phillips, James B DoD A f US USA MEDCOM CDMRP; Gordon Broderick
Cc: Fisher, Rebecca A Dr CTR US USA MEDCOM CDMRP; Strock, Abigail I Ms CIV USA MEDCOM USAMRAA
Subject: RE: Report Disapproved - DOD Award (10-1-0774) Review of ANNUAL REPORT GW093042 (UNCLASSIFIED)

Classification: UNCLASSIFIED
Caveats: NONE

Good Morning-

It looks like this research doesn't end until 2013, if that's the case I think it's best to wait until closer to the end date to request an extension.

Please let me know if you have any questions.

Thank You,
Abby

Abigail Strock
abigail.l.strock@us.army.mil
301-619-2342
Appendix B: CV of Candidate Research Associate
**Objective**

Postdoctoral Associate

**Summary of Qualifications**

8 years of experience teaching at the undergraduate level, and 5 years in the research field of theoretical and computational biophysics, including 7 publications to date. Performed both research and lecturing duties while studying at the graduate level. Reputation for clear and concise explanations of difficult and novel concepts both in writing and teaching. Outstanding work ethic, with a dependable and approachable demeanor, naturally produces scientific collaboration. Committed to physics advancement through education and original research.

**Summary of Skills**

Molecular Modeling (PyMol, Modeller, VMD)
Molecular Dynamics Simulations (Amber, Gromacs, NAMD)
Docking (Autodock, Dock)
Binding Site Prediction (PASS, LIGSITE, Q-SiteFinder, SURFNET, Metapocket)
Programming Languages (C, FORTRAN, Python, Shell Script)
Mathematical Programming (Maple, MATLAB)

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Curriculum Vitae T.J.A. Craddock

Education

UNIVERSITY OF ALBERTA, Edmonton, AB
Doctoral Degree, In progress (candidacy passed August 24, 2009), expected completion Fall 2011
  • Degree Area: Theoretical Condensed Matter Physics (Biophysics)
  • Working Thesis Title: The Physical Basis for a Nanoneuroscience of Memory

UNIVERSITY OF ALBERTA, Edmonton, AB
Master’s of Science Degree, June 2008
  • Degree Area: Theoretical Condensed Matter Physics (Biophysics)
  • Thesis Title: An Assessment of the Information Processing Capabilities of Microtubules at Physiological Temperature

UNIVERSITY OF GUELPH, Guelph, ON
Bachelor of Science Degree, February 2002
  • Degree Area: Honors Physics
  • Co-op option completed

Teaching Experience

GRANT MACEWAN UNIVERSITY, Edmonton, AB
Sessional Instructor
  Supervisor: Shelley Lorimer
    • Graded seminar sets, laboratory reports, midterm and final exams

UNIVERSITY OF ALBERTA, Edmonton, AB
MASC Exam Review Instructor
  Supervisor: Carmen Ropchan
    • Prepared review session material including example problems, practice problem sets and solutions for “Physics 124 – Particles and Waves”, “Physics 130 - Wave Motion, Optics and Sound”, “Physics 230 - Electricity and Magnetism”, and “Engineering Physics 131 – Engineering Dynamics”
    • Instructed review sessions using prepared material for groups of 70-80 students

UNIVERSITY OF ALBERTA, Edmonton, AB
High School Physics Session Instructor
  Supervisor: Isaac-Yakoub Isaac
    • Instructed laboratories, and performed demonstrations for high school students
    • Aided and assisted students in performing lab experiments
CURRICULUM VITAE T.J.A. Craddock

UNIVERSITY OF ALBERTA, Edmonton, AB

Teaching Assistant
Supervisor: Isaac-Yakoub Isaac

- Taught laboratory portion of “Physics 144 - Newtonian Mechanics and Relativity”, "Physics 146 - Fluids and Waves”, and “Physics 230 - Electricity and Magnetism”
- Graded laboratory reports, and lab exams

UNIVERSITY OF ALBERTA – AUGUSTANA, Camrose, AB

Sessional Laboratory Instructor
Supervisor: Gerhard Lotz

- Instructed laboratory portion of “Augustana Physics 110 - Mechanics”
- Graded laboratory reports

UNIVERSITY OF GUELPH, Guelph, ON

Teaching Assistant
Supervisor: Ernie. McFarland

- Taught tutorial and laboratory portion of “Physics 1000 – An Introduction to Mechanics”, "Physics 1020 - Introductory Physics”, and “Physics 1010 – Introductory Electricity and Magnetism”
- Graded laboratory reports, created and graded quizzes, and graded exams

Professional Experience

UNIVERSITY OF ALBERTA, Edmonton, AB

Research Assistant
Supervisor: Jack A. Tuszynski

- Performed research relevant to the projects of Professor Tuszynski
- Assisted in the preparation and submission of publications for Professor Tuszynski

UNIVERSITY OF GUELPH, Guelph, ON

Research Assistant
Supervisor: Gabriel Karl

- Performed research relevant to the projects of Professor Karl

TRIUMF LABORATORY, Vancouver, BC

Research Assistant
Supervisor: Lutz Moritz

- Transported and modified legacy code for radiation safety software
- Developed a genetic algorithm to analyze neutron spectra
ONTARIO POWER GENERATION, Toronto, ON  May – August 2000

Developmental Student
Supervisors: Crawford Morrison, and Herminia Roman
- Performed static and dynamic code testing on radiation safety software
- Created software design documents for radiation safety software

ONTARIO POWER GENERATION, Toronto, ON  September – December 1999

Developmental Student
Supervisors: Crawford Morrison, and Herminia Roman
- Performed static and dynamic code testing on radiation safety software
- Developed a method of manual code implementation to test software

ONTARIO HYDRO - NUCLEAR, Pickering, ON  January – April 1999

Developmental Student
Supervisors: Jeff Weed, and Don Jarron
- Performed Y2K compliance testing on software
- Performed static and dynamic analysis on safety code

Research Interests

To understand how the nervous system operates, how it processes information to bring about various actions, by applying new nanoscale techniques to uncover the cellular and molecular bases of cognitive phenomena, such as perception, learning, memory, language, thinking, attention, and awareness. This “Nano-neuroscience” approach has potential to progress the biophysical understanding of neurological, neuropsychiatric and neurodevelopmental diseases, such as Alzheimer's disease, Frontotemporal Dementia Parkinson's disease, and Huntington’s disease, and provide novel avenues for their treatment and prevention. General topics include: Psychoactive compound interactions with the neuronal cytoskeleton, Biomolecular memory storage mechanisms, Bionano information processing mechanisms, Bioelectronics and Coherent energy transfer

Teaching Interests

Cellular and Molecular Biophysics  Electricity and Magnetism
Nanoneuroscience
Undergraduate physics

Awards, Scholarships and Grants

J. GORDIN KAPLAN AWARD
University of Alberta  April 2011
- Awarded to assist in the exposure of student research results and conclusions to peer review, and to test ideas with acknowledged experts.
Curriculum Vitae T.J.A. Craddock

**PRESIDENT’S DOCTORAL PRIZE OF DISTINCTION**
University of Alberta September 2010

- Awarded in recognition of academic and scholarly achievement to those holding a major award.

**NSERC POSTGRADUATE SCHOLARSHIP - DOCTORAL**
Natural Sciences and Engineering Research Council of Canada May 2009 – April 2011

- Awarded to outstanding Canadian students at the Doctoral level pursuing a degree in natural sciences or engineering.

**PRESIDENT’S DOCTORAL PRIZE OF DISTINCTION**
University of Alberta September 2009

- Awarded in recognition of academic and scholarly achievement to those holding a major award.

**GSA PROFESSIONAL DEVELOPMENT AND TRAVEL GRANT**
University of Alberta September 2009

- Granted to allow participation in such academic development activities as conference, research trips, professional development, and so forth.

**QUEEN ELIZABETH II**
Province of Alberta September 2008 – April 2009

- Formerly the Province of Alberta Graduate Fellowship
- Awarded to student of Canadian citizenship on the basis of academic achievement.

**BILLY MILLS AWARD**
University of Alberta December 2007 & January 2009

- Awarded to student who is active in the Aboriginal community, of Aboriginal ancestry, with good academic achievement

**GRADUATE STUDENT SCHOLARSHIP**
Province of Alberta December 2006

- Awarded for exceptional academic achievement in the first year of a Master's program
DOROTHY LESLIE MEMORIAL AWARD
University of Alberta

December 2006

- Awarded to student with satisfactory academic standing who is of Aboriginal descent

MARIE LOUISE IMRIE GRADUATE STUDENT AWARD
University of Alberta

April 2006

- Awarded to assist in the exposure of student research results and conclusions to peer review, and to test ideas with acknowledged experts.

UTS GRADUATE STUDENT TEACHING AWARD
University of Alberta

March 2006

Honorary

- Awarded for excellence in teaching

UNIVERSITY GRADUATE SCHOLARSHIP
University of Guelph

September 2002

- Awarded for academic achievement

REGISTRAR’S ACCESS AWARD
University of Guelph

September 1997

- Awarded for academic merit

Accepted Publications


**Manuscripts Submitted and In-preparation**


**Conference Presentations**


T.J.A. Craddock, D. Friesen, and J.A. Tuszynski, Investigating Quantum Mechanisms of Energy Transfer in Microtubules (poster), University of Alberta 1st Annual Symposium on Graduate Physics Research, Edmonton, Canada, September 17th, 2010


**Workshop Presentations**


**Technical Reports**

Validation Exercise 1 Report for ORIGEN-S (version SCALE 4.2), Ontario Power Generation Internal Document VE-REP-ORIGEN, August 2000


**Community Involvement**

**Student Government**

CONDENSED MATTER PHYSICS REPRESENTATIVE, University of Alberta Graduate Physics Student Association
September 2008 – August 2011
COMMITTEE MEMBER, University of Alberta Physics Graduate Curriculum Committee
September 2008 – August 2009

Mentoring

MENTORING ABORIGINAL PEERS, University of Alberta Aboriginal Student Center
September 2008 – April 2009

EDMONTON BRAIN BEE, University of Alberta Outreach Program
February, 2008

WP WAGNER MENTORSHIP PROGRAM, University of Alberta Outreach Program
November 2006 - June 2007

AVONMORE SCHOOL EXPERTS DAY, University of Alberta Outreach Program
February 2006

Community Outreach

ST. MARKS AND AVONMORE SCHOOL SCIENCE FAIR
University of Alberta Outreach Program
March 2006

Professional Affiliations

American Physical Society
Association for the Scientific Study of Consciousness

References

DR. JACK A. TUSZYNSKI, PHD
Professor, Physics & Oncology, University of Alberta
Allard Research Chair, Cross Cancer Institute
MSc. and PhD. Advisor
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Email: jackt@ualberta.ca

DR. STUART R. HAMEROFF, MD
Professor Emeritus, Anesthesiology & Psychology, University of Arizona
Director, Center for Consciousness Studies
Scientific Collaborator
Phone: (520) 626-5605
Email: hameroff@u.arizona.edu
Dr. Nancy J. Woolf, PhD  
Professor, Psychology, University of California, Los Angeles  
Scientific Collaborator  
Phone: (310) 206-7874  
Email: nwoolf@ucla.edu

Dr. Shelley Lorimer, PhD, P.Eng  
Professor and Chair, Engineering, Grant MacEwan University  
Supervisor  
Phone: (780) 497-4631  
Email: lorimers@macewan.ca

Carmen Ropchan, MSc.  
Director, Math & Applied Sciences Centre, University of Alberta  
Supervisor  
Phone: (780) 492-6272  
Email: cropchan@ualberta.ca