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TITLE:High-Fidelity Design of Multimodal Restorative Interventions in Gulf War Illness

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14. ABSTRACT

Our objective is to further refine models of immune and endocrine regulatory dysfunction developed under W81XWH-10-1-0774 (Broderick PI) by improving fidelity of the timescale and drug action thereby translating previously idealized treatments into optimally beneficial low-risk drug re-purposing strategies that are immediately deployable as short exposure courses in phase-I clinical trials.

With collaborating PI Dr. Whitley (CSU), we continue to make substantive progress towards project goals during this reporting period, specifically in the development of i) a formal algorithmic approach for direct integration of data with the contextual logic, ii) an algorithmic approach for model discovery and validation, and iii) redefinition of the treatment paradigm to focus on destabilization of illness and remission "reachability".

15. SUBJECT TERMS
GWI; Hypothalamic-pituitary-adrenal (HPA) axis

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1. Introduction

Gulf War Illness (GWI) is a complex illness with symptoms presenting across several principal regulatory systems including immunologic and endocrine components. Accordingly, we proposed that GWI might involve a chronic imbalance in co-regulation between the nervous, endocrine and immune systems. We tested this hypothesis under our previous CDMRP award W81XWH-10-1-0774 by using a discrete logic formalism to construct a first set of computer models describing the interactions between regulation of stress hormones (hypothalamic-pituitary-adrenal; HPA axis), sex hormones (hypothalamic-pituitary-gonadal; HPG axis) and immune function in the periphery and the brain. Results of our first analysis (**Craddock et al., 2014**] suggested that normal homeostatic drive across the immune, sex and stress hormone regulatory systems may contribute to the persistence of GWI by naturally supporting an alternate steady state characterized by chronically high cortisol, low testosterone and a shift towards Th1 immune activation. Further work has shown that subtle changes to the circuitry itself can produce exact overlap of alternate homeostatic signature with GWI in a set of readily observable immune and endocrine markers (**Rice et al., 2014**). This involvement of a modified homeostatic drive implies that once activated, this regulatory program will actively resist therapeutic attempts to restore normal hormone and cytokine levels. This regulatory dynamic must be overcome and ideally exploited to therapeutically escape what is essentially a stable disease state in GWI.

Computer simulations using these initial models predicted very low success rates for single-target treatment strategies. Only when endocrine and immune components were targeted together and in a specific sequence did predictions of sustained remission reach favorable levels. One such two-step strategy involves inhibition of Th1 pro-inflammatory signaling (e.g. short course Enbrel), followed by blockade of glucocorticoid receptors (e.g. Mifepristone) once the system has equilibrated (**Craddock et al., 2015**). However this initial proof-of-concept work assumed that intervention agents were bound to target instantaneously with ideal affinity and specificity. While these *idealized* treatments helped identify targets for which novel high affinity drugs could be developed, *the current award supports the continued refinement of this computational platform to (i) directly incorporate the pharmacologic properties of drugs available for repurposing and (ii) better represent the time course dynamics of regulatory signaling and drug action kinetics*. These enhancements will support the translation from idealized treatment strategies into predicted real-world treatment courses that make optimal use of currently available drugs.

2. **Keywords**: dynamical systems, discrete logic models, immune endocrine regulation, system models, constraint satisfaction, stable attractors, limit cycles

3. Accomplishments.

What were the major goals of the project?

In this first year of the project efforts were focused on elements that were supportive of the following goals:

- *Specific Aim 1*: Implement relative dynamics.
  Milestone #1: Completion and release of validated model incorporating updated state transition dynamics, deployed using a novel time and structure-based decomposition scheme.
  Estimated % complete: 70%

- *Specific Aim 2*: Incorporate available drug action data.
  Milestone #2: Completion and release of validated model incorporating estimates of drug-action dynamics.
  Estimated % complete: 20%

- *Specific Aim 3*: Increase efficiency of search for optimal intervention course.
  Milestone #3: Completion and release of validated model decomposition scheme and hybrid treatment optimization algorithm for rapid deployment and efficient use of large-scale distributed platform.
  Estimated % complete: 60%

What was accomplished under these goals?

From a technological perspective, recall from the annual report for Year 1 that we have significantly re-engineered the basic structure of the model in favor of a generalized discrete framework introduced by Thomas and colleagues (**1991, 1995, 2001**). In this formalism every element (gene, protein, cell, etc…) can assume a number of discrete states proportional to the number of control interactions it exerts on other elements in the regulatory network., Each of these control actions only becomes available above a specific expression *threshold*. Furthermore, the relative strength of promoters and inhibitors are captured by a set of *tunable* logical operators (*K*) making it possible to reproduce their net combined control action in a specific context.

In this second year, work has focused on exploiting this much more flexible framework by developing, implementing and testing formal algorithmic approaches for directly integrating experimental data in 1) the tuning of the contextual logic and corresponding discovery of gaps in the regulatory circuitry, 2) automated assembly of large-scale networks form literature, 3) the formal reduction of unobservable segments of the model (multi-resolution capability) and 4) the development of treatment designs that exploit the plasticity of the stability landscape.

1. *Direct integration of experimental data into regulatory logic tuning and model validation*. This effort directly supports the completion of Major Task 1, creating an architecture that not only integrates observations at stable resting states seamlessly but also formally exploits the exact temporal sequence of transition states. The inclusion of transition states in the tuning of model logic greatly improves fidelity and talks full advantage of available time course trajectories such as experimental data documenting immune (18 cytokines and soluble mediators) and endocrine response to exercise in healthy and GWI veterans collected under previous CDMRP award W81XWH-09-2-0071 (Klimas PI). Specific initiatives include the following:

To implement this and complete *Subtask 1.c and 1.d*, as well as support *Subtask 3.a and 3.c*, we fundamentally redesigned our approach to model tuning and validation. Specifically, we have moved away from the previous iterative assessment the statistical proximity of predicted and observed stable states using Brown's multiple test method (**Brown, 1975**). We propose instead a formal we propose a Constraint Satisfaction (CS) based frame-work for parameter identification in these large multi-valued regulatory networks that directly integrates observed system behavior. Under this approach:

- The regulatory networks and their corresponding dynamics are translated into multi-valued logical equations. Then these equations along with specific observations and more general biologically inspired constraints are formulated as a Constraint Satisfaction (CS) problem (**Corblin et al., 2012**) in support of *Subtask 3b*. The central principle is that all measured and postulated states must be *reachable* through state transitions (single or multiple) supported by the model circuitry and logic transition rules. The computational efficiency of this approach has been dramatically improved through work by Dr. Whitley's group at CSU.
- Importantly these constraints can be formulated to include and be tolerant of uncertainty with respect to the presence and exact nature of a given control action as well as sparse availability of samples (very small data sets) and incompletely observed state profiles (i.e. only a minority of species were measured in a given sample).
- The resulting CS problem is solved with a fast clause hybrid solver known as *Chuffed* (**Ohrimenko et al. 2009**). Models satisfying the constraints of the data and overall stability (or multi-stability) are then ranked on the basis of additional properties, inspired by the work of **Klarner et al.** (**2012**) and driven forward by work with **Collaborating PI, Dr. Whitley**. Consistent with *Subtask 3b*, in its current iteration as a multi-objective problem, our model ranking scheme estimates the ideality of a parameter set that satisfies the reachability of known states by further assessing and favoring:
    I. *Parsimony* of the circuit architecture or the minimal circuit connectivity necessary for the model to satisfy the data constraints,

II. Kinetic *efficiency* of signaling or a minimal number of transitions bridge between any 2 known states, and
III. Outcome *robustness* or the uniqueness of a desired target state given an initial system state.
IV. *Maximally actionable* signal specificity or maximizing the number of distinct control actions with the minimal number of activation thresholds.

Working in close collaboration with Dr. Whitley's team at CSU we are now exploring the most appropriate relative weighting of signal robustness and kinetic efficiency by computing these measures in known biological circuits.

- *Flexible discovery of control actions*. By applying these constraints and optimality measures the algorithm proposes changes to the control actions in the initially proposed regulatory network, suggesting deletion of some superfluous edges and the inclusion of new edges that would improve adherence to the constraints. These edits are limited to areas of the network that have been identified a priori as having shallow evidence and low confidence.

- *Validation of parameter estimation in several updating schemes*. In support of *Subtask 1d and 3c*, this proposed framework was evaluated against 4 well-known biological regulatory networks: the hypothalamic-pituitary-adrenal (HPA) axis (**Ben-Zvi et al., 2009; Gupta et al., 2007**), the In vivo Reverse engineering and Modeling Assessment (IRMA) network (**Bockmayer et al., 2012**), a Mammalian cell cycle (**Faur et al., 2006**) and a Dendritic cell cycle (**Garg et al., 2008**). The results of our implementation of CS are summarized in **Table 1**. Listed are the number of elements and density of control interactions in each of these benchmark biological networks. For each network, we report the number of competing parameter sets identified that satisfy the data constraints equally well under fully synchronous updating, fully asynchronous updating as well as *priority update with memory* (P. Memory). Results show that our CS framework is capable of working efficiently with relatively detailed (>100 nodes), densely connected (~40%) networks that are severely under-constrained, yielding over 3M competing parameter sets in less than 30 minutes on a single processor laptop.

2. *Automated assembly large-scale network models*. This increase in computational efficiency makes it possible to significantly increase the scale and resolution of the networks that can be analyzed. For example, in our previous proof of concept work under CDMRP award W81XWH-10-1-0774, mainframe computers were used to explore the regulatory programs supported by a network consisting of 21 aggregate molecular/cellular functional nodes interacting through 78 directed edges using a basic fixed logic (**Fritsch et al, 2013**). The latter was assembled over the course of 6 months by manual curation of close to 100 journal papers. The current generation of methods support the analysis on a single processor of networks consisting of hundreds of nodes interacting through several hundred control actions. *This scale exceeds the reach of manual curation*. To address this dramatic increase in scale we have partnered with Elsevier Life Science Solutions (ELSS) to jointly develop text mining tools capable of extracting regulatory relationships from several million whole text journal publications. Results from natural language processing with MedScan (**Novichkova et al., 2003**) are re-interpreted and validated further using an application of *context-sensitive* sentiment analysis being developed by our group under Python's NLTK library. Disagreements between MedScan and sentiment-based interpretations are flagged for targeted expert review. So far these have numbered less than 20% of the regulatory connections extracted from text. In collaboration with ELS, our group is assembling training reference sets to support machine learning of the optimal weighting of modifying words to support more reliable and accurate interpretation. Extracted network interactions are then assigned a *confidence score* by our group based on the *credibility* of the source and the *clarity* of the statement supporting the proposed regulatory interaction.

   We are currently applying this rapid prototyping of larger and more comprehensive models to:

   - Assemble a higher resolution model of endocrine-immune regulation in *female physiology*. A recent iteration of a female HPA-HPG -immune axis model now includes the hypothalamic pituitary thyroid (HPT) axis capturing metabolic repercussions of the illness. The full model was

assembled from automated text mining of 34,078 journal papers and consists of 53 entities (nodes) connected initially by 674 control actions (edges) (**Figure 1**). For each of these control actions, validation was conducted across 2 NLP platforms. Text excerpts extracted and interpreted by MedScan were re-interpreted by our own sentiment analysis as described above. Of the original 676 control actions 512 achieved consensus across platforms, agreeing on source to target direction and mode of action (inhibit or promote). Interpretation was divergent across platforms in 162 control edges (close to 1 in 4). Manual review of the underlying citations by domain experts supported assignment of 134 of these control actions, leaving 28 as ambiguous even to the human expert (or < 5%) (**Figure 2**).

We are currently tuning this model to circadian rhythm and menstrual cycle, and will also be using exercise data collected from female veterans under CDMRP award GW150199 (Craddock PI).

- Create a pathway level model of intracellular signal transduction in close collaboration with Drs. O'Callaghan, Vrana and group with an initial variant serving to support a recent CDMRP submission (Oct. 2017) directed at identifying key signaling phosphoproteins and subsequent pathway perturbations responsible for the neuroinflammatory effects of GW-relevant exposures as targets for therapeutic intervention. This preliminary model demonstrates a rapid prototyping capability of seamlessly working at multiple levels of biology. The basic model was assembled by automated text mining of 5,154 journal publications capturing 206 regulatory interactions linking 59 key proteins across the principal signal transduction pathways (**Figure 3**). Early simulations applying binary logic to this network produced alignment in 5 of the 7 proteins interrogated experimentally in mouse cortex exposed to DFP with prior administration of corticosterone in pilot data produced by the O'Callaghan team at CDC/ NIOSH. This model is now being tuned to additional data as to becomes available and served as a proof-of-principle model in support of a recent application by O'Callaghan and Broderick labs at CDC/ NIOSH and RGH.

3. *Adjustable resolution through formal network reduction*. Although the candidate regulatory networks and corresponding parameter sets identified using constraint satisfaction can also serve to estimate missing data elements, incomplete data profiles rapidly inflate the candidate solution space and the corresponding computational burden. In an attempt to counter this, we have been examining approaches for adjusting the level of model detail such that state space of the model more closely aligns with the space measured experimentally while still also supporting the same dynamic regulatory behavior. In a first step the direction and mode of control actions are rationalized to create the reduced network which is more observable. In the current iteration this is applied to the reduction of sink nodes and simple mediators only, that is elements with single input and single output. Following this structural rationalization, then in a second step the lumped control logic is tuned to available data such that the reduced network is capable of reproducing the same dynamic behaviors as the fully expanded network. The next phase of development will formally include the network dynamic behavior in the iterative structural rationalization instead of having these as sequential steps.

4. *Destabilizing persistent illness through directed treatment*. Our initial approach to treatment design was directed at repeated large-scale simulations with virtual trials being iteratively designed, evaluated and incrementally improved by a flexible global search algorithm. With developments in the model paradigm the focus has shifted from a simulation-intense strategy to one focused on *reachability* of the remission state from an initial stable illness state. This too has evolved from a simple search for efficient and robust paths that make remission *reachable* to one that also accounts for the *plasticity* of the attractor landscape. Recent work has focused on a study of how inclusion of exogenous elements like a stressor, a pathogen or a drug actually serve to augment the basic regulatory circuit. This new endogenous-exogenous hybrid circuit is different from the native circuit and may support completely new programs and/ or favor existing regulatory programs at the expense of others. The implication is that every new drug-target combination must now be treated as a new circuit with its own emergent state transition graph. This is shift in paradigm from a directed walk along the same fixed state transition network.

In an example of this we show the state transition graph (STG) for the native HPA axis in the absence of any environmental perturbations in **Figure 4A**. The STG in the lower panel shows the sequence of states that the HPA axis must occupy given the biological circuitry illustrated in the top panel. The unperturbed HPA axis supports 2 oscillatory stable states, one at the high end of cortisol (CORT) expression and one at the lower end of the range. These are distinct and one cannot be reached from the other. Under the effects of a stressor applied to CRH, these available oscillatory programs collapse to 2 sets of attractors depending on an individual's perception of stress (sensitive or relatively insensitive)(**Figure 4B**). If the impact of stress on CRH expression is muted then the system will either galvanize the healthy cycle or transition to a stationary point located on the low cortisol cycle. In the case of a high sensitivity of CRH to stress, the regulatory programs available collapse to 3 stationary points, 2 of which are located in the low or hypocortisolic realm suggesting that in a biologically noisy environment one would have twice as many chances of remaining trapped in a low cortisol regulatory program once the stress abates. In **Figure 4C** we show that if a glucocorticoid receptor (GR/ R) blocker is applied the entire attractor landscape is altered with the low cortisolic cycle becoming dynamically unstable and all states converging towards the higher range attractor.

5. *Towards incorporating drug action data into the simulation model.* This observation whereby the inclusion of a drug does not simply guide transition along a fixed of path but instead transforms the stability landscape is now directing our design of an approach to high throughput screening of treatment options. In support of *Major Task 2*, we are working towards a multi-tiered scenario where a large-scale drug-target database would be used in a first tier to construct a broad set of all alternative drug-augmented circuits. Towards this end, in conjunction with work completed under DoD GWIRP awards W81XWH-16-1-0632 (Craddock PI) and W81XWH-16-1-0552 (Craddock PI) Research Programmer I, Rajeev Jaundoo, amalgamated numerous drug-gene databases into one large database. The databases currently include the pharmacogenomics knowledgebase (PharmGKB), FDA's National Drug Code Directory (FDA NDC), DrugBank.ca, the toxin and toxin-target database (T3DB), Guide to Pharmacology (GtP), and the HUGO Gene Nomenclature Committee (HGNC). MATLAB scripts were created that allow future versions of each of the above datasets, and new databases to be easily appended to the consolidated database. After raw database information is downloaded, the amalgamation script parses the file to obtain information relevant to the dataset, and includes any combination of gene-drug-target interactions. This relevant information is then written to an output file and added to the overall dataset. Other supplementary databases such as ReaxSys Medicinal Chemistry, and SciFinder are being explored. Each of the corresponding drug-target interactions would then be translated into their corresponding state transition graphs and reachability analysis conducted to verify that the healthy attractor is *reachable* by the drug-augmented circuit. Positive instances of predicted reachable remission would then be assessed at a higher resolution in a second tier using ongoing work in high-throughput drug docking and prediction of drug action underway in the Craddock lab. Specifically the Craddock group is using a quorum across a combination of molecular dynamic software platforms (including Glide and Vina) to produce higher resolution estimated probabilities of binding for the FDA approved drug candidates from tier 1. Currently computed values include binding energy, and Probability of Not Binding to target. These parameters describe binding alone; the mode of drug action as an agonist or antagonist must still be derived from published pharmacodynamics literature. We are continuing to work closely with Dr. Craddock's group to extend beyond proximal binding to include transport to target processes e.g. absorption, diffusion. Avenues being considered may be informed at least in part by earlier work from **Ridgway and Broderick (2008)**

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

The Broderick group at RGH is currently reviewing terms to host visiting scholars in Rochester supported by Elsevier Life Science Solutions.

How were the results disseminated to communities of interest?

Ongoing progress continues to be shared with fellow researchers and with veteran advocates through the meeting schedule established for the Gulf War Illness Research Consortium. In addition Drs. Broderick and

Craddock continue to deliver podium talks and participated in discussion panels. Specifically, Dr. Broderick's work was profiled in the Elsevier Connect electronic magazine and his talk webcast to a broad internal audience at Elsevier in March, 2017.

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

Ongoing work will now focus increasingly on i) continued development of our revised approach to treatment design which will be directed towards massively parallel assessment of competing drug-target networks and ii) the integration into the simulation space of the predicted drug action spearheaded by the Craddock group. The focus will be on *reachability* of the remission state, as well as the notion that treatment remolds the attractor landscape, essentially *destabilizing* the illness state.

4. **Impact**.

What was the impact on the development of the principal discipline(s) of the project?
In keeping with the milestones described in the project submission initial accomplishments that have impacted the core disciplines of the project may be summarized as follows:

- In an effort driven by the CSU team, *we were able to implement a very computationally efficient approach for seamlessly integrating data with the causal regulatory circuit*. We are currently conducting tests on actual and simulated biological networks consisting of over 1000 control actions with the identification of parameter sets occurring within 60 minutes on a laptop (Subtask 1.c, 3.a and 3.c).
- Together with the CSU team *we have devised and are testing additional metrics for ranking competing models.* Measures such as signaling efficiency and robustness are being used to study known biological networks and will be instrumental in defining optimality of treatment.
- Keeping pace with this increase in the scale of problems that we can now analyze efficiently, we have partnered with Elsevier and have designed and deployed our own *natural language processing* (NLP) tools for improving the reliability of networks assembled from text-mining of literature. Currently we are continuing to build on this relationship to integrate the ReaxSys Medicinal Chemistry database.

What was the impact on other disciplines?

The development of this computational framework has broad reaching applications beyond the model-based design of treatments for Gulf War Illness. The resulting physiological models can be used to examine and study any complex illness and we are currently exploring concurrent opportunities to automate the generation of new model segments that might add resolution to specific other illnesses, for example, the Broderick group has recently expanded the scope of its work to include the study of host-pathogen interactions and the design of vaccine therapies.

What was the impact on technology transfer?

This work has attracted the attention of Elsevier research and we are examining opportunities to collaborate on the automated generation of new models from Natural Language Processing of medical texts.

What was the impact on society beyond science and technology?

Nothing to report.

5. **Changes/Problems**:

Changes in approach and reasons for change
In February of 2017, Dr. Broderick moved his research group from NSU to the Rochester General Hospital in upstate New York where he now leads a new hospital-based research center

(https://www.rochesterregional.org/CCSB/). Senior staff members, Hooman Sedghamiz, Mark Rice, and Saurabh Vashishtha migrated with Dr. Broderick and now work from this new Center in Rochester which is co-directed by Dr. Sol Efroni, a distinguished cancer systems biologist at Bar Ilan University (Israel).

Rochester Regional Health has a strategic partnership with Rochester Institute of Technology and Dr. Broderick also holds an appointment as Research Associate Professor in the Department of Biomedical Engineering. These joint appointments offer an unprecedented access to engineering and computer science students as well as the ability to supervise their graduate studies. The new Center also has close ties to the State University of New York (SUNY) at Buffalo and the Buffalo Clinical and Translational Research Center which houses state of the art high-performance computing and broad omics cores.

Actual or anticipated problems or delays and actions or plans to resolve them
In order to avoid any interruptions in research progress under this and other related awards:

a) Dr. Broderick elected to leave the award at NSU and Dr. Craddock has graciously assumed the responsibilities of acting PI.

b) Dr. Broderick's new home institution (Rochester Regional Health) has accepted to waive all indirect costs and align benefit rates such that the project budget is not adversely affected by this move. This has been applied to all related projects that were underway with Dr. Broderick at NSU.

With the opening of the new Center at RGH and the ratification of sub-awards we expect no further changes in project logisitcs.

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

Significant changes in use or care of human subjects	Nothing to report
Significant changes in use or care of vertebrate animals.	Nothing to report
Significant changes in use of biohazards and/or select agents	Nothing to report

6. **Products**.
The results of the latest analyses are being communicated as follows:

- We currently have an advanced draft of a manuscript describing the revised logic formalism and expect to be submitting this for publication in the coming quarter.
- The Craddock group submitted the following for publication

  Jaundoo R, Bohmann J, Gutierez G, Klimas NG, Broderick G, Craddock TJA. Using a Consensus Docking Approach to Estimate the Sociability of FDA-Approved Drugs to Predict Repositioning, Polypharmacology and Side-Effects. Scientific Reports. 2017, Under Review.

7. **Participants & Other Collaborating Organizations**:

This past year has seen the Broderick group's interactions with Elsevier Life Sciences intensify with new collaborative research in the automated assembly of large-scale regulatory models and more recently developer access to their medicinal chemistry database.

What individuals have worked on the project?

Name	Travis Craddock
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	12
Contribution to Project:	Dr. Craddock has taken over administrative leadership of the project as PI with the relocation of Dr. Broderick to RGH. Primary responsibility and leadership of drug selection and drug property modeling component of the project
Funding Support:	No change

Name	Gordon Broderick
Project Role:	Co-PI
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	12
Contribution to Project:	Works closely with Dr. Craddock in the overall direction of project and coordination of efforts. Primary responsibility and leadership of model logic, logic tuning and treatment course redesign initiatives.
Funding Support:	No change

Name	Mark Rice
Project Role:	Research Programmer II/ Grant-funded research staff
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	12
Contribution to Project:	Oversees all programming initiatives as they apply to large-scale computation. Conducted migration of previous model paradigm from C into MatLab. Now leads the automated text mining component
Funding Support:	Fully supported

Name	Hooman Sedghamiz
Project Role:	Research Programmer II/ Grant-funded research staff
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	8
Contribution to Project:	Oversees all model algorithm development. Has spearheaded the theoretical re-assessment of the basic logic model, greatly improving the versatility and fidelity.
Funding Support:	Fully supported

Name	Mary Ann Fletcher
Project Role:	Co-Inv
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	0.6
Contribution to Project:	Continues to coordinate the processing samples and delivery of endocrine and immune data from exercise challenge that is being

	used in the tuning of the model logic
Funding Support:	No change

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

In the past year Dr. Craddock's 2 new CDMRP awards (GW150199, GW150144) have become active. He serves as PI (4.8 months) with Dr. Broderick as Co-investigator (2.4 months).

What other organizations were involved as partners?

There are no partnering organizations at this time other than Colorado State University.

8. **Special Reporting Requirements**: None

Collaborative Awards. This work is being carried out in collaboration with Dr. D. Whitley at Colorado State University under the associated grant number W81XWH-15-1-0583.

Abbreviations. Abbreviations found in the HPA-HPG-HPT-immune model of **Fig. 1, 4**

ACTH	Adrenocorticotropin hormone;
AR	Androgen Receptor
AVP	Vasopressin;
CRH	Corticotropin-releasing hormone;
ER	Estrogen Receptor
FSH	Follicle-stimulating hormone;
FST	Follistatin;
GCR (R)	Glucocorticoid receptor
GH1	Growth hormone 1;
GNRH1	Gonadotropin releasing hormone 1;
GNRH2	Gonadotropin releasing hormone 2;
IFNG	Interferon gamma;
IGF1	Insulin-like growth factor 1;
IgG,	Immunoglobulin gamma;
IL-2	Interleukin 2
IL2-R	IL–2 receptor
INS	Insulin;
KISS1	Kisspeptin;
NO,	Nitric oxide;
NK cell,	Natural killer cell;
OXT	Oxytocin;
PTGS2	Prostaglandin-endoperoxide synthase 2;
SST	Somatostatin;
TGFB1	Transforming growth factor beta 1;
TNF	Tumor necrosis factor;
TNFSF13B	Tumor necrosis factor superfamily member 13B/BAFF;
TSH	Thyroid stimulating hormone;
TNFa	Tumour necrosis factor
TNFaR	TNFa receptor
Th17,	T-helper 17 cell;
Th2,	T-helper 2 cell;
Th1,	T-helper 1 cell;
Treg,	Regulatory T cell
TRH	Thyrotropin-releasing hormone;

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Table 1. *Comparative assessment of Constraint Satisfaction tuning*. Summary of the number of elements and density of control interactions in 4 well-documented biological regulatory networks. For each model the number of competing parameter sets that satisfied the data constraints equally well is reported for fully synchronous, fully asynchronous and priority update with memory.

Network	Nodes	Edges	$\lvert K \rvert$	Nr. Identified parameters			Time taken (in sec)		
				Synch	Asynch	P. Memory	Synch	Asynch	P. Memory
HPA Axis	4	6	46,656	1	16	1	0.023	0.164	0.045
IRMA	6	9	$\approx 604 \times 10^6$	23,294	3,276800	NA	30.004	1225	NA
Mammalian	10	39	$27,607 \times 10^{66}$	819,200	204,800	NA	1386	1805	NA
Dendritic Cell	111	124	$95,268 \times 10^{135}$	102,400	51,200	NA	1798	1800	NA

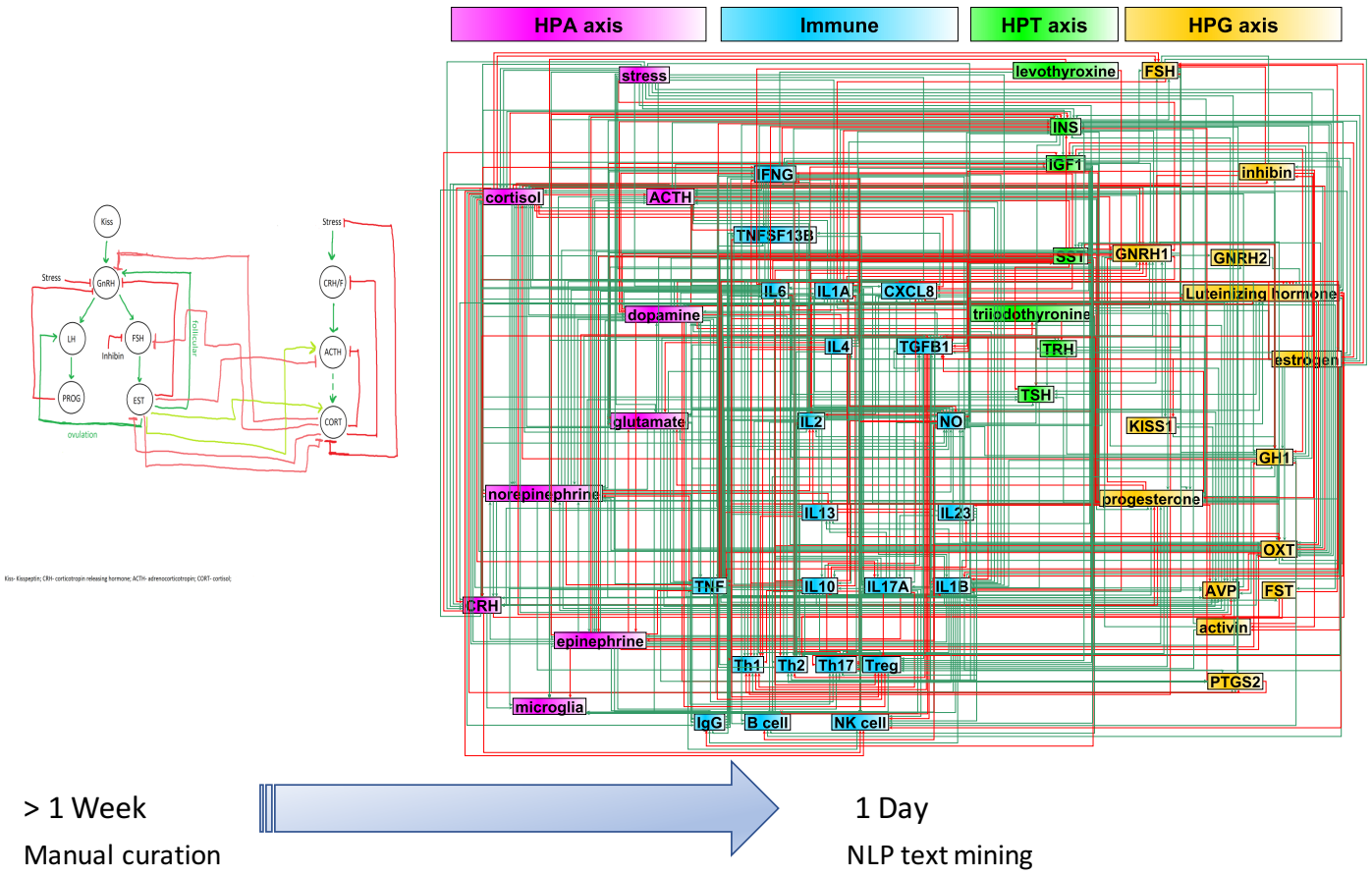

Figure 1. *Automated assembly of large-scale regulatory networks*. A partial model of the female hypothalamic-pituitary-gonadal (HPG) and adrenal (HPA) axes assembled manually over the course of 1 week consisting of 9 species (nodes) linked by 19 control actions (edges) based on manual review of fewer than 50 journal papers (panel **A**). A broad model bridging across hypothalamic-pituitary-gonadal (HPG), adrenal (HPA) and thyroidal (HPT) interaction with adaptive and innate immunity (panel **B**). The latter consists after rationalization, filtering and partial tuning of 52 cellular/ molecular entities (nodes), linked by 630 control actions (edges) supported by citations from 33,110 full journal texts.

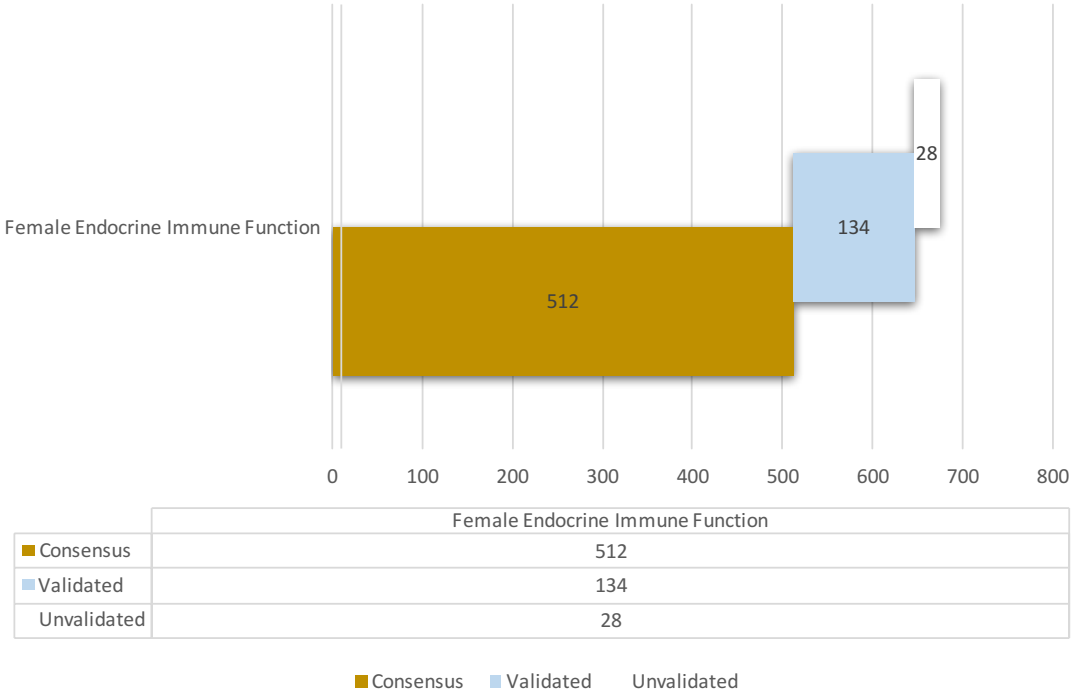

Figure 2. *Network verification using a consensus scheme*. Breakdown of consensus and divergence on the verification of control actions across 2 natural language processing (NLP) platforms: MedScan (Novichkova et al., 2003) and our own context sensitive sentiment analysis (Rice, Sedghamiz et al., in preparation).

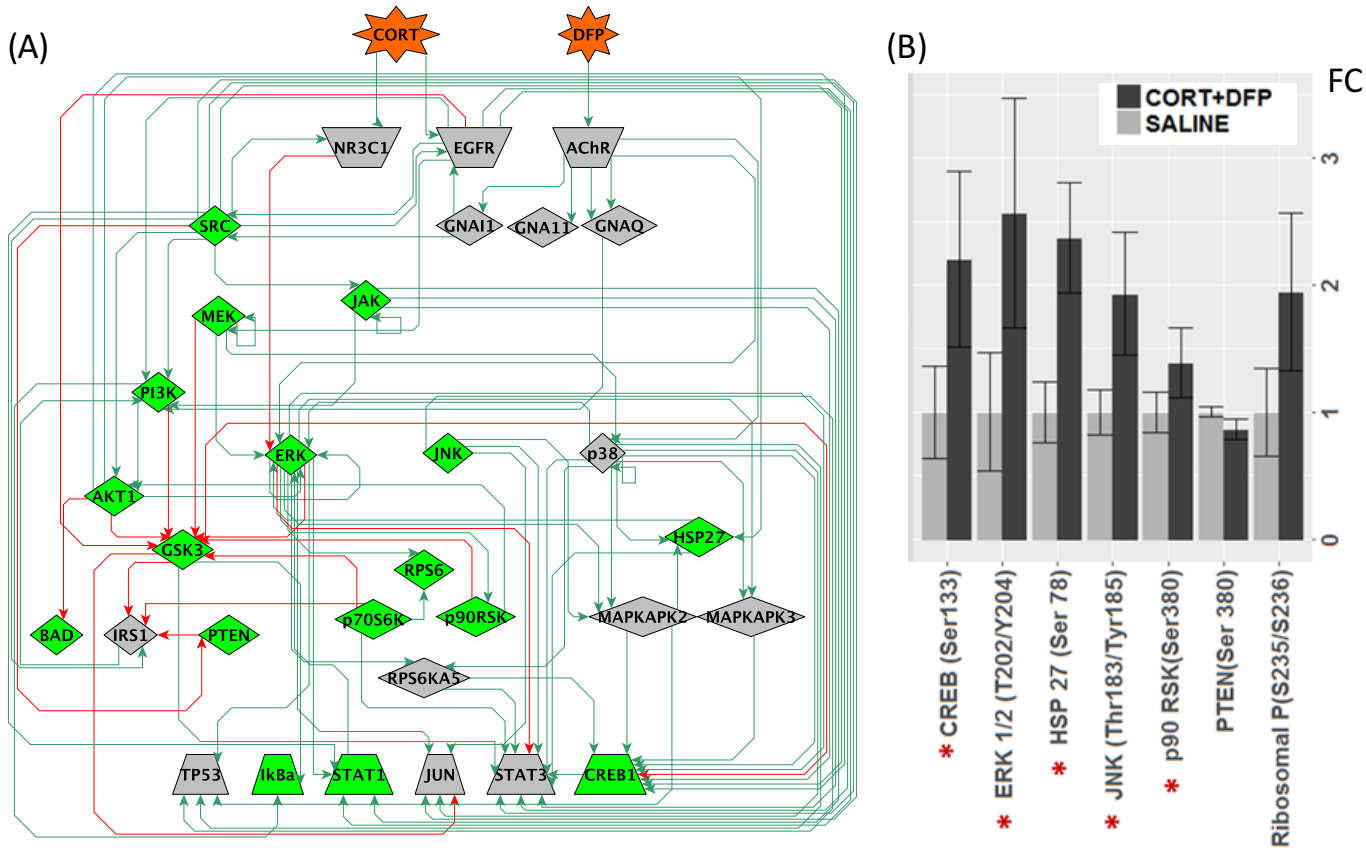

Figure 3. *An Immune Signal Transduction Model of Stress Potentiated Response to DFP.* A causal map of stimulatory and inhibitory molecular signals along a subset of core pathways involved in response to corticosterone (CORT) and the neurotoxin DFP (panel **A**). Interactions were identified by text mining the broad literature database available as part of the Pathway Studio suite (Elsevier Life Science Solutions, Rockville MD). Predictions from this partial preliminary model show broad alignment experimental data (panel **B**), agreeing with expression changes in 5 out of 7 proteins (red star *) measured during acute response to DFP exposure preceded by corticosterone administration. We expect tuning of the model logic to the prosed new data to further improve fidelity and predictive capability.

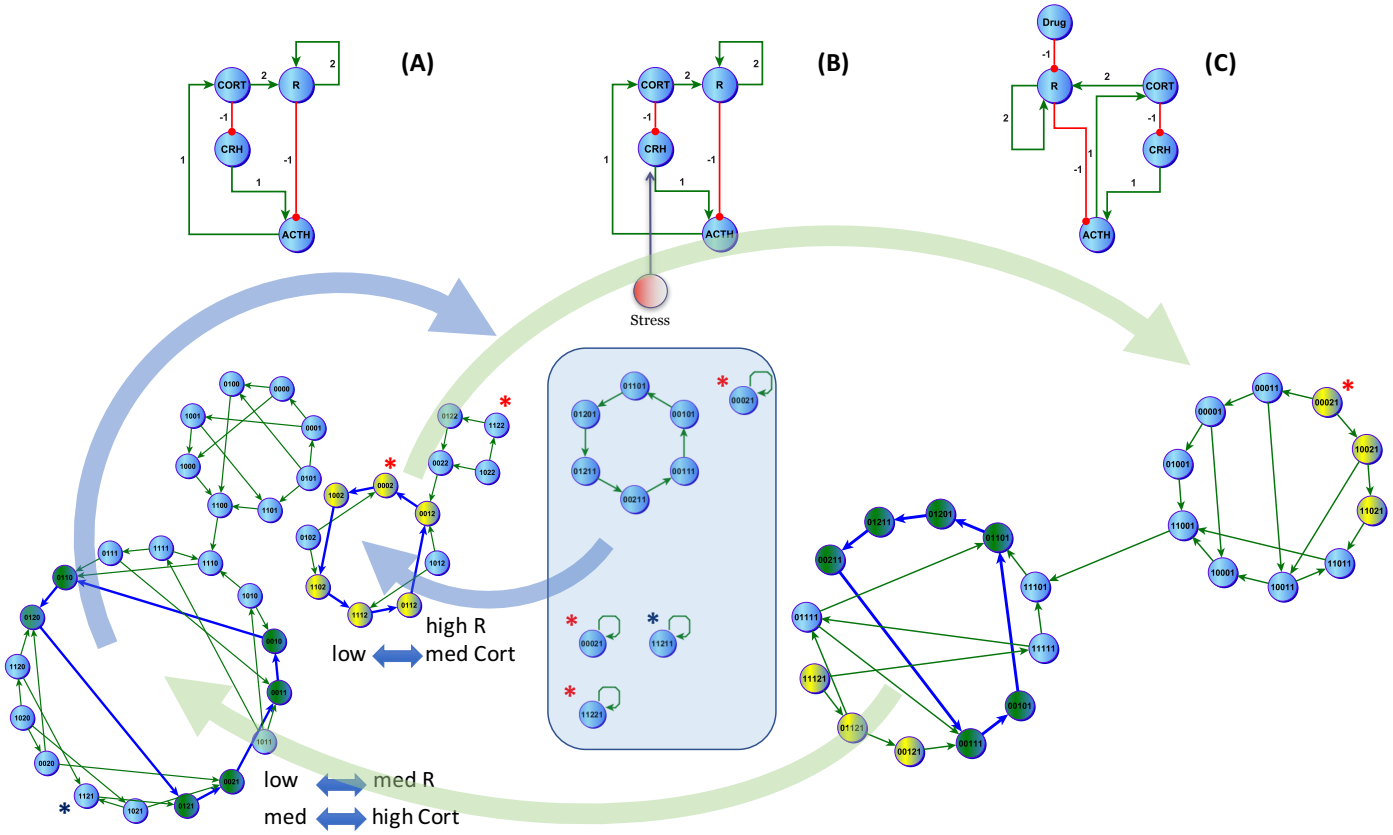

Figure 4. *Treatment paradigm revisited*. Circuit diagram of the basic HPA axis (top 3 circuits) at rest (panel **A** at left), under sustained stress (panel **B** center), and under the actions of a glucocorticoid (GR) blocker (panel **C** right). Below each circuit are the ordered state transitions, or state transition graph (STG), describing the behavior that the circuit can support or must adhere to. At rest the systems supports 2 stable distinct and unreachable oscillatory programs, namely low and high range cortisol cycles. Applying stress to the CRH node collapses these cycles in favor of 2 sets of states including normally unstable states based on *sensitivity* or degree of responsiveness (high in bottom set and low in top set) to stress.