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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Individuals with myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) complain of profound fatigue. We here performing a series of experiments to understand the central nervous system mechanism of fatigue. We are studying a new candidate fatigue-mediating neuron type, which expresses the neuropeptide NPVF and which is located in the dorsomedial hypothalamus (DMH). Our first objective is to test the role of *Npvf* neurons in fatigue behavior. Our second objective is to develop an ELISA assay to detect NPVF in bodily fluids. The achievement of these two objectives opens the door to future directions aimed at understanding fatigue in general and specifically in individuals with ME/CFS. We have made progress in both objectives. We have shown that activation of the Npvf neurons causes a reduction in wheel running behavior, a proxy for fatigue. We have also shown that mice lacking the neuropeptide NPVF resume wheel running behavior after lipopolysaccharide injection earlier than control mice.

15. SUBJECT TERMS
Fatigue, sleep, mouse, NPVF, neuropeptides, ELISA

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1. Introduction

Fatigue not refreshed by sleep is a cardinal symptom of mylagic encephalomyelitis/ chronic fatigue syndrome (ME/CFS), a prevalent disease in both the military and civilian population. Peripheral immune dysregulation may trigger fatigue, but brain mechanisms of fatigue remain under-studied. We propose to use the **PRMRP Discovery Award** to study a novel central nervous system mechanism of fatigue. While fatigue is a subjective complaint, there are behavioral consequences to fatigue, which can be studied in experimental systems. These include reduced physical activity, reduced motivation for reward, and increased sleepiness. Sickness-induced fatigue in humans is often associated with immune cell activation. In animals we can experimentally activate the immune system using bacterial cell wall components and, thereby, trigger fatigue.

2. Keywords

Fatigue, sleep, mouse, NPVF, neuropeptides, ELISA,

3. Accomplishments

Major task 1: test the role of mouse NPVF neurons in LPS-induced fatigue

Sub task 1: use a chemogenetic approach to test whether NPVF neuron activation induces reduced voluntary wheel running behavior and increased sleep.

We have shown that chemogenetic activation of NPVF neurons reduces voluntary wheel running (VWR) behavior in male mice (**Fig. 1**).

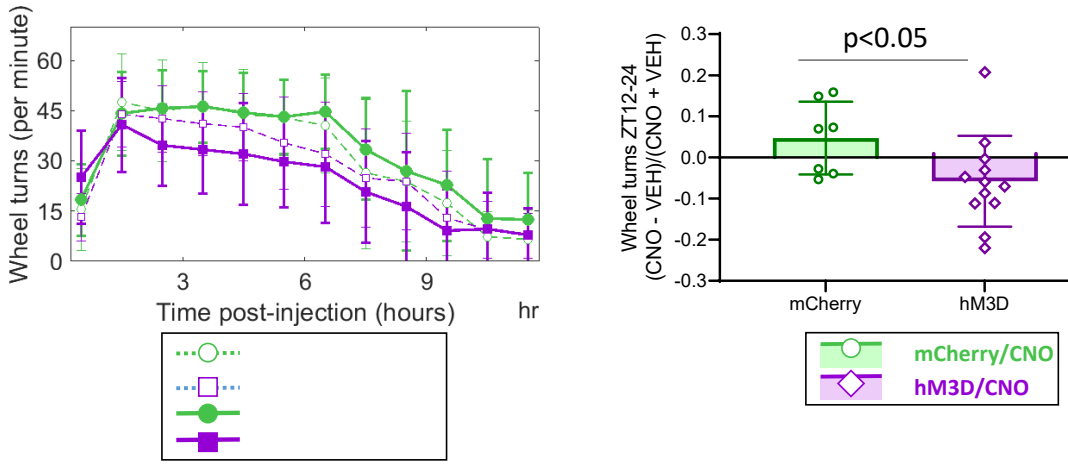

Figure 1: reduced voluntary wheel running in the 12 hours after IP injection of CNO into npvf^{CRE} male infected with AAV carrying CRE-dependent hM3D-mCherry of mCherry male mice.

We have also tested whether *npvf* knockout mice show attenuated suppression of VWR behavior in response to lipopolysaccharide (LPS) injection. Our data show accelerated recovery from LPS injection in npvf knockout female mice (**Fig. 2**).

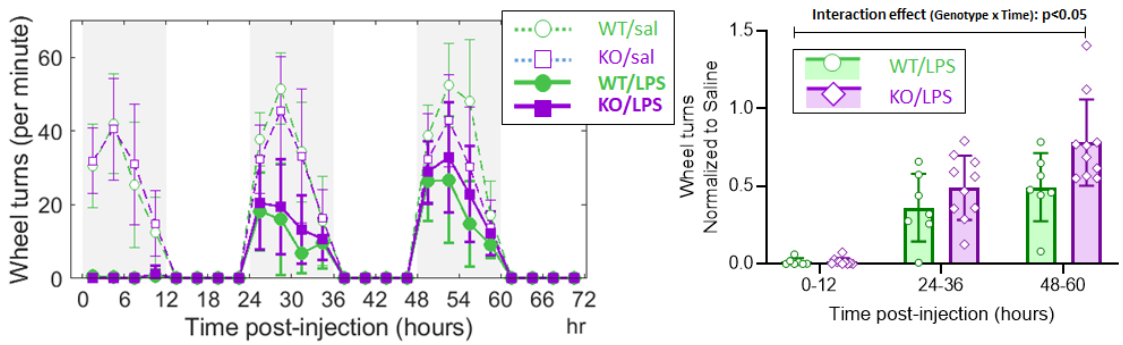

Figure 2: Accelerated recovery of voluntary wheel running in the 3 days after IP injection of lipopolysaccharide (LPS) into npvf(ko) female mice. Comparison is shown for wild-type parental C57B6/j control female mice.

The above two experiments both support our over-arching hypothesis. We are currently repeating the npvf knockout experiment, this time in both females and males.

We are currently trouble shooting our mice with npvf-CRE transgene, which appears to have been silenced (see below “Problems” section.)

We are not yet ready to report these results at meetings or in manuscripts.

Major Task 2: develop an ELISA for detecting NPVF in bodily fluids

We have been using an ELISA to test the sensitivity and specificity of commercially available antibodies raised towards human NPVF-derived peptides. We are also testing an anti-avian GnIH (gonadotropin Inhibitory Hormone), which is the bird homolog of mammalian NPVF. We have not yet tried a sandwich ELISA.

4. Impact

The proposed research application concerns the FY21 Department of Defense Health Program CDMRP Funding Opportunity W81XWH-21-PRMRP-DA. Area of Encouragement is Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS), with special emphasis on two areas of critical need:

(1) Research to understand the mechanisms underlying ME/CFS, and

(2) Research to identify biomarkers to diagnose and test potential therapeutics for ME/CFS.

The critical gap in our knowledge of ME/CFS that the proposed research program will attempt to fill is an absence of a brain mechanism for fatigue, the cardinal symptom of ME/CFS. The proposal outlines a series of experiments designed to test the function of a novel neuroanatomical substrate mediating fatigue behavior. These are the neurons in the dorsomedial hypothalamus (DMH) expressing the neuropeptide VF (NPVF). If the proposed experiments support the over-arching hypothesis, the impact would be the discovery of a new mechanism for fatigue.

While we trigger fatigue behavior in our studies using acute peripheral immune activation, in future studies we will study DMH neurons in murine conditions of chronic fatigue. Moreover, we will use the ELISA we develop as part of this Discovery Award to begin measuring NPVF levels in bodily fluids of patients with ME/CFS and other conditions of chronic fatigue.

5. Changes/Problems

We initially saw robust CRE-dependent gene expression in our NPVF-IRES-CRE mice (and we were able to generate the data shown in figure 1 above).

However, we have recently found severely reduced CRE expression in the mice we generated with the IRES-CRE recombinase knocked into the 3’UTR of the *npvf* gene. We no longer see expression of CRE-dependent transgenes packaged into AAV viral vectors. We have also performed quantitative reverse transcriptase PCR and observe about a 10,000-fold reduction in CRE expression in comparison to CRE expression in a control VGAT:CRE mouse.

We have checked the sequence of CRE and the surrounding DNA in which it was inserted and found no mutations. We therefore believe we are witnessing epigenetic silencing of this transgene. We are currently working with the Penn transgenic mouse core facility in an attempt to identify the reason for the silencing of the CRE. We plan to back-cross our npvf-CRE mice to C57B6/j parental mice. In parallel, so as to minimize time used, we will be asking our transgenic core facility to generate a new mouse with IRES-CRE knocked into the npvf locus.

This unanticipated problem has slowed progress in this project. In addition, there were delays in hiring staff (Keelee Pullum) to perform experiments.

6. Products

None

7. Participants & Other Collaborating Organizations

Name	Role in project	ORCID ID	Nearest person month worked	Contribution	Funding support
David Raizen	PI	0000-0001-5935-0476	1.0	Project director	This award
Allan Pack	Co-I		0.6	Co-investigator	This award
Michael Iannacone	Graduate student		2.35		T32 pre-doctoral fellowship
Keelee Pullum	technician		7		This award

8. Special Reporting Requirements

None

9. Appendices

None