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PRINCIPAL INVESTIGATOR: Donald M. Kuhn

CONTRACTING ORGANIZATION: Wayne State University, Detroit, MI

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Kuhn, Donald M.

E-Mail: donald.kuhn@wayne.edu

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Department of Psychiatry and
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**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**
The hypothesis under investigation in this **Tier 1 Discovery** application is that the symptom clusters of GWI are a manifestation of a dysbiosis of the gut microbiome. The primary symptoms of GWI, which have been validated in the most recent (2016) report by the Committee on Gulf War and Health, are mood alterations (depression and anxiety) and gastrointestinal dysfunction (gut inflammation, leakiness). While these symptoms may not seem linked or linkable, they can easily be accounted for by a GWI-induced disturbance in the gut microbiome. Each of the symptoms of GWI, separately and as studied in non-GWI clinical conditions, can result from a dysbiosis of the microbiome but the possibility that this occurs in GWI has not been investigated. Therefore, there is a clear and compelling rationale for determining how communication along the gut-brain axis is disrupted in GWI. The Topics of Special Interest being addressed in this proposal are: a) gastrointestinal abnormalities as a component of GWI; b) molecular signatures (e.g., biomarkers) underlying symptom clusters via genomic, proteomic, metabolic, or epigenetic technologies; and c) dysregulation of, or abnormal crosstalk between, human body organ systems (e.g., neuroinflammation, autonomic dysfunction) and in particular, neurological system, immune system and endocrine, exocrine and/or excretory systems (e.g., gastrointestinal) as they apply to the gut-brain axis. This application has been informed extensively by **The Gulf War Illness Landscape** as suggested in the GWIRP-IIFRA Program Description.

**15. SUBJECT TERMS**
None listed.

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|---|---|---|---|---|---|
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## TABLE OF CONTENTS

## 1. INTRODUCTION:

This project investigated how Gulf War Illness (GWI) changes the gut microbiome using a validated animal model of this condition. The purpose was to determine how the structure and composition of the microbiome is altered by GWI and then use microbiota transfer therapy (MTT) from normal mice to normalize the GWI-altered gut microbiome in treated mice.

## 2. KEYWORDS:

Gulf War Illness, gut microbiome, microbiota transfer therapy, permethrin, pyridostigmine bromide, anxiety, depression, GI inflammation, GI leakiness, dysbiosis.

## 3. ACCOMPLISHMENTS:

**What were the major goals of the project?**

Specific Aim 1: To characterize gut microbiome dysbiosis in a mouse model of GWI
Major Task 1: Treat mice with GWI modeling compounds (i.e., pyridostigmine (PG) and permethrin (Per).
Major Task 2: Characterize gut microbiome in controls and treated mice using 16S rRNA next generation
Status: Specific Aim 1 and all major tasks completed.

Specific Aim 2: Rebalance GWI-induced gut dysbiosis using probiotics and microbiota transfer sequencing and liquid chromatography/mass spectrometry.
Major Task 3: Rebalance dysbiosis using probiotics
Major Task 4: Rebalance dysbiosis using microbiota transfer (fecal transplantation)
Status: The work on this specific aim and the major subtasks has been completed and the data analysis is underway and will be completed in several weeks.

**What was accomplished under these goals?**

1) Major activities: Mice were treated with the GWI agents permethrin (PER) plus pyridostigmine bromide (PB) for 10 days according to published accounts. Mice were then given a 6 month rest period for the symptoms of GWI to evolve. Controls received the vehicles for PER and PB on the same schedule as the experimental group. Each group was divided into 2 equal groups and fecal pellets were collected for preparation of solutions for microbiota transfer therapy (MTT). The GWI mice will be donors for FMT into controls and controls will be donors for MTT into the GWI group.
2) Specific objectives: The goals of this experiment are to transfer the GWI-modified gut microbiome using MTT into control mice to determine if the recipient mice express the symptoms and transfer a normal or control gut microbiome using MMT into GWI-treated mice to determine if the symptoms of GWI are reduced.
3) Key outcomes: Fecal pellets were collected from all mice in both groups (GWI and control) and subjected to 16S rRNA sequencing monthly to monitor the evolution of changes in the gut microbiome caused by PER + PB. It was noted that the emergence of symptoms of GWI tracked with alterations in the gut microbiome, which is in keeping with our hypotheses. The MTT procedure has been carried out and the mice have been given a rest period to allow for colonization of their gut microbiome with the donor gut microbiome. Initial results indicate that the cross transfer MTT was successful- the control recipients from GWI donors are developing symptoms and the GWI recipients from control donors are showing symptom improvement.
4) Other achievements: The goals of these experiments have been met and the project has arrived at the end of the funding period.

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

Nothing to report.

**How were the results disseminated to communities of interest?**

Publications:

Angoa-Perez, M., Zagorac, B., Francescutti, D.M., Winters, A.D., Greenberg, J.M., Ahmad, M.M., Manning, S.D., Gulbransen, B.D., Theis, K.R. and Kuhn, D.M. Effects of a high fat diet on gut microbiome dysbiosis in a mouse model of Gulf War Illness. Scientific Reports, 10 (1):9529. doi: 10.1038/s41598-020-66833-w, 2020. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7293234/pdf/41598_2020_Article_66833.pdf

Angoa-Perez, M., Zagorac, B., Francescutti, D.M., Theis, K.R. and Kuhn, D.M. Effects of gut microbiota remodeling on the dysbiosis induced by high fat diet in a mouse model of Gulf War Illness. Life Sciences, Special Issue on State-of-the-Science of Gulf War Illness, 279, 119675, 2021. https://www.sciencedirect.com/science/article/pii/S0024320521006615?via%3Dihub

Poster presentations:

Kuhn, Donald M. Effects of a high fat diet on gut microbiome dysbiosis in a mouse model of Gulf War Illness. 2020 Virtual Gulf War Illness (GWI)-State of the Science Conference, August 18-19, 2020.

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

Nothing to report- this is a final report.

## 4. IMPACT:

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

Perhaps the most relevant and important impact of these studies was the finding that an animal model of GWI confirmed a significant alteration in the gut microbiome. This finding supports our hypothesis that a dysbiosis in the gut microbiome may underlie the symptoms of GWI. The three most significant symptom clusters of GWI- GI disturbances, CNS alterations (anxiety, depression, PTSD) and chronic fatigue) can each be cause individually by an alteration in the gut microbiome. It was therefore reasoned that all of these symptoms can be caused by a dysbiosis in the gut microbiome. In addition, the ability to influence the course of GWI (improve or worsen) by using MTT to transfer a gut microbiome from a diseased mouse to a healthy one, and vice versa, will open new avenues of investigation targeting the gut microbiome as a therapeutic target in GWI. Based on the growing number of publications and funded grants that are now studying the gut microbiome, our original contributions to this field have had a positive impact.

**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:

**Changes in approach and reasons for change**

There were no changes in the approach, objectives or scope on this project.

**Actual or anticipated problems or delays and actions or plans to resolve them**

A slight delay was encountered as a result of the impact of the Covid 19 pandemic. Our labs are in a VAMC and we were deemed to be essential employees so all of our laboratory personnel were permitted to continue research operations. The interruptions caused by the pandemic were minor and temporary.

**Changes that had a significant impact on expenditures**

There were no changes or events that had a significant impact on expenditures.

**Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

**Significant changes in use or care of human subjects**

Not applicable.

**Significant changes in use or care of vertebrate animals.**

None to report. All of our laboratory care technicians reported to work during the pandemic and the ordering and care of animals continued uninterrupted.

**Significant changes in use of biohazards and/or select agents**

No changes to report.

## 6. PRODUCTS:

- **Publications, conference papers, and presentations**

**Journal publications.**

Publications so far (others will follow):

Angoa-Perez, M., Zagorac, B., Francescutti, D.M., Winters, A.D., Greenberg, J.M., Ahmad, M.M., Manning, S.D., Gulbransen, B.D., Theis, K.R. and Kuhn, D.M. Effects of a high fat diet on gut microbiome dysbiosis in a mouse model of Gulf War Illness. Scientific Reports, 10 (1):9529. doi: 10.1038/s41598-020-66833-w, 2020. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7293234/pdf/41598_2020_Article_66833.pdf. Federal support acknowledged.

Angoa-Perez, M., Zagorac, B., Francescutti, D.M., Theis, K.R. and Kuhn, D.M. Effects of gut microbiota remodeling on the dysbiosis induced by high fat diet in a mouse model of Gulf War Illness. Life Sciences, Special Issue on State-of-the-Science of Gulf War Illness, 279, 119675, 2021. https://www.sciencedirect.com/science/article/pii/S0024320521006615?via%3Dihub Federal support acknowledged.

**Books or other non-periodical, one-time publications.**

None.

**Other publications, conference papers, and presentations**.

* Kuhn, Donald M. Effects of a high fat diet on gut microbiome dysbiosis in a mouse model of Gulf War Illness. 2020 Virtual Gulf War Illness (GWI)-State of the Science Conference, August 18-19, 2020.

- **Website(s) or other Internet site(s)**

None to report.

- **Technologies or techniques**

None to report.

- **Inventions, patent applications, and/or licenses**

None.

- **Other Products**

None to report, other than publications and conference presentation.

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

**What individuals have worked on the project?**

| Name | Role | Person months | Contribution | Funding |
|---|---|---|---|---|
| Donald M. Kuhn | PI | 2.4 | Unchanged from previous report | This award |
| Mariana Angoa-Perez | Co-investigator | 1.8 | Unchanged from previous report | This award |
| Kevin R. Theis | Collaborator | 1.2 | Unchanged from previous report | This award |
| Brian Gulbransen | Collaborator | 1.0 | Data analysis, bioinformatics, manuscript prep, revision, response to reviewer comments | Not funded on this award |
| Shannon Manning | Collaborator | 1.0 | Data analysis, bioinformatics, manuscript prep, revision, response to reviewer comments | Not funded on this award |

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

Nothing to report for the PI.

Co-investigator Mariana Angoa-Perez, PhD, received the following grant support. This grant was not submitted as of the time of funding of the present award:
Project Number: GW200036
Name of PD/PI: Mariana Angoa-Perez
Source of Support: Department of Defense
Primary Place of Performance: Wayne State University
Project/Proposal Start and End Date: 07/2021 – 06/2024
Total Award Amount (including Indirect Costs):
Person Months (Calendar/Academic/Summer) per budget period. 0.2

**What other organizations were involved as partners?**

Nothing to report. Remains the same as in previous report.

**8. SPECIAL REPORTING REQUIREMENTS**

**COLLABORATIVE AWARDS:**

**QUAD CHARTS:**

**9. APPENDICES:**

A. Journal article (10 pages)
B. PI CV (47 pages)

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# Effects of gut microbiota remodeling on the dysbiosis induced by high fat diet in a mouse model of Gulf war illness

Mariana Angoa-Pérez [a,b,*], Branislava Zagorac [a,b], Dina M. Francescutti [a,b], Kevin R. Theis [c,d], Donald M. Kuhn [a,b]

[a] Research and Development Service, John D. Dingell VA Medical Center, Detroit, MI, USA
[b] Department of Psychiatry and Behavioral Neurosciences, Wayne State University School of Medicine, Detroit, MI, USA
[c] Department of Biochemistry, Microbiology and Immunology, Wayne State University School of Medicine, Detroit, MI, USA
[d] Perinatal Research Initiative in Maternal, Perinatal and Child Health, Wayne State University School of Medicine, Detroit, MI, USA

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ABSTRACT

Gulf war illness (GWI) is a chronic disorder of unknown etiology characterized by multiple symptoms such as pain, fatigue, gastrointestinal disturbances and neurocognitive problems. Increasing evidence suggests that gut microbiome perturbations play a key role in the pathology of this disorder. GWI courses with gut microbiota alterations and their metabolites (e.g. short chain fatty acids -SCFA-), which can be aggravated by lifestyle risk factors such as a high fat diet (HF). To investigate the causative role of the gut microbiome, non-absorbable antibiotics (Abx) were administered to mice treated with GWI agents and concomitantly fed with a HF. In light of the wide use of Abx as pseudo-germ-free models, we evaluated the effects of Abx exposure on GWI and HF on body weight, food intake, gut microbiota changes and levels of the SCFA acetate. Results show that HF decreased food intake while increasing body weight in both controls and GWI. Exposure to Abx prevented these HF effects by offsetting the body weight gain in GWI. GWI and HF led to decreases in α-diversity, disruptions in the composition and structure of the gut bacterial community and decreases in acetate levels. This Abx-induced remodeling of the gut microbiome was characterized by an expansion of Proteobacteria, decreases in Bacteroidetes and Firmicutes, and overall increases in acetate levels, as well as by the proliferation of potential pathobionts. Therefore, the use of Abx may not represent a dependable approach to deplete the gut microbiome and its advantages as a pseudo germ-free model warrant further investigation.

## 1. Introduction

Gulf war illness (GWI) is a chronic multisymptomatic disorder of unknown etiology characterized by a variety of symptoms such as pain, fatigue, gastrointestinal disturbances and neurocognitive problems [1]. The lack of widely accepted outcome measures complicates the study of GWI and highlights the need for identification of therapeutic targets. The influence of microbiota on host physiology has gained attention in the context of GWI and an increasing number of studies point to perturbations in the gut microbiome as key players in the pathology of this disorder [2–6]. Preclinical studies in rodents found that exposure to GWI agents caused a gut microbiome disruption, termed dysbiosis, that produced a significant decrease in intestinal tight junction proteins, leading to a leaky gut, activation of enteric glial cells, inflammation and endotoxemia [3,7,8]. In humans, a recent pilot study reported significant gut bacteria alterations in Veterans diagnosed with this disorder [9], with these gut microbiome differences extending to those individuals with GWI who also presented with gastrointestinal disturbances compared to those who did not display intestinal symptoms [9]. Dietary regimens, including those rich in fat and carbohydrates are among the most influential environmental factors with the capacity to induce gut microbiome dysbiosis [10,11]. In this sense, a cross-sectional survey conducted in a cohort of 15,000 GWI Veterans reported that nearly 50% were overweight and about 30% were obese [12], with those being obese being more prone to develop other chronic health conditions such as post-traumatic stress disorder [12]. Reports in mice have shown that although treatment with GWI agents per se does not lead to being overweight, consumption of a high fat diet (HF) post-GWI agent exposure leads to a significant increase in body weight [2] and metabolic alterations [5]. These outcomes are associated with a gut

* Corresponding author.
*E-mail address:* maperez@med.wayne.edu (M. Angoa-Pérez).

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microbiome dysbiosis characterized by a reduction in bacteria which produce short chain fatty acids (SCFAs) [5]. SCFAs (e.g. acetate, propionate and butyrate) are produced via fermentation of indigestible dietary carbohydrates and fiber by the gut microbiota [13]. It has been shown that an exogenous application of SCFAs can prevent weight gain in HF-induced obese mice and overweight humans [14,15]. Given that the microbiota-related alterations in GWI can be aggravated by HF, we sought to evaluate the causative role of the gut microbiome. In an attempt to remove the intestinal microbiota as a regulatory mechanism, a cocktail of non-absorbable antibiotics (Abx) was administered to mice treated with GWI agents and concomitantly fed a HF. Abx have been widely used to generate pseudo-germ-free rodents as an alternative model for proof-of-principle studies [16], and in the present work, the effects of Abx exposure were evaluated on GWI and HF on body weight, food intake, gut microbiota remodeling and acetate levels.

## 2. Materials and methods

### 2.1. Subjects, Gulf war illness agents, diets and antibiotics

The present study employed an extensively validated GWI model and administration of a high fat diet (HF) as previously described [2]. Briefly, 2–4 male C57BL6/J mice (8 weeks of age purchased from Envigo) were housed per cage in a room with constant temperature and humidity and with alternating 12 h periods of light and darkness. All mice used in these studies were from the same cohort and assignment to treatment groups was random, with at least 2 cages per group to avoid cage effects. Half of the mice were injected with 50 μl of GWI agents in final doses of 0.7 mg/kg of pyridostigmine bromide (PB) and 200 mg/kg of permethrin (PER) solubilized in dimethyl sulfoxide (DMSO) to a final DMSO concentration of 3% just prior to intraperitoneal injection. The other half served as controls and received intraperitoneal injections of 3% DMSO in sterile physiological saline. Injections were administered once daily for 10 days. During treatment with GWI agents, mice were given ad libitum access to water and standard rodent laboratory chow ((ND); LabDiet 5001 containing 28.5% protein, 13.5% fat, and 58% carbohydrates). On the last day of treatment, the GWI and control groups were split into 4 same sized groups ($N = 6–7$ mice per group) and fed the following diet regimens: two groups on a ND and two groups on a HF (D12451, Research Diets with 20% protein, 45% fat, and 35% carbohydrates). Sample sizes were based on our previous study showing an effect of this HF diet on GWI-treated mice [2]. An antibiotics cocktail (Abx) or vehicle solution was administered in the drinking water simultaneously to the two diets for 21 days. Fresh solutions were prepared every other day and administered in graduated glass bottles (Braintree Scientific) containing sipper tubes with ball bearings to minimize loss of fluid to drippage. This cocktail consisted of ampicillin trihydrate (0.25 g/ml), neomycin trisulfate (0.25 g/ml), metronidazole (0.25 g/ml), and vancomycin hydrochloride (0.125 g/ml), all purchased from Sigma Aldrich, in 3% sucrose to encourage drinking. These are broad-spectrum antibiotics without systemic effects due to their poor absorption (neomycin, is not absorbed at all). Control mice received only a 3% sucrose solution, which was found to have no impact on the gut microbiome (see Supplementary Fig. 1 data). Hereafter, the groups are referred to as Con_ND −Abx, Con_ND +Abx, Con_HF −Abx, and Con_HF +Abx for controls and GWI_ND −Abx, GWI_ND +Abx, GWI_HF −Abx, and GWI_HF +Abx for PER + PB treated mice.

Food and fluids intake, as well as body weights were recorded every 2–3 days throughout the experiment. Fluid intake was determined by weighing each bottle at the start of the test period and subtracting their weights after 24 h. Consumption for each mouse was normalized to body weight and presented as g of consumed food or fluid/ g of body weight/ 24 h period. Mice were sacrificed by decapitation and the contents of the caecum were harvested and frozen at −80 °C. The Institutional Care and Use Committee of Wayne State University approved the animal care and experimental procedures (IACUC 17-08-0307). All procedures were also in compliance with the *NIH Guide for the Care and Use of Laboratory Animals*, with ARRIVE guidelines and under IACUC-approved protocols.

### 2.2. Gut microbiome analysis

16S rRNA genes in the caecum were sequenced as reported previously [2,17]. In brief, bacterial DNA was extracted and purified using the QIAamp PowerFecal DNA Kit. The V4 hypervariable region of the bacterial 16S rRNA gene was amplified using dual indexed, Illumina compatible primers and the library was loaded onto an Illumina MiSeq standard V2 flow cell for sequencing in a 2 × 250 bp paired end format. The raw 16S rRNA gene sequences from the paired fastq files were processed with the Divisive Amplicon Denoising Algorithm (DADA2) pipeline (v 1.12.1) to obtain merged, denoised, chimera-free, inferred amplicon sequence variants (ASVs) suitable to identify fine-scale variation [18]. ASVs were defined by 100% sequence similarity, and analyzed using DADA2 in R (v 3.6.2), according to the online MiSeq protocol (https://benjjneb.github.io/dada2/tutorial.html), with some modifications that included truncation lengths of 240 bp and 160 bp and a maximum number of expected errors of 2 bp for forward reads and 5 bp for reverse reads. Sequences were classified using the "silva_nr_v132_train_set" database after removal of sequences derived from Archaea, Chloroplast, or Eukaryota as previously described [19]. ASVs count were calculated for each group. Gut microbiome α-diversity was characterized using the Chao1 (i.e. community richness), Shannon and Simpson (1-D) (i.e. community heterogeneity) indices, and data were thereafter visualized and statistically analyzed with GraphPad Prism (v 9.1). Microbial β-diversity was assessed using the Jaccard (i.e. shared composition) and Bray-Curtis (i.e. shared structure) indices based on ASV relative abundance data in R. High-dimensional class comparisons were carried out with LEfSe in an on-line interface [20], using default parameters with the exception that the LDA score was set to 3.6. Taxonomic classifications of ASVs from the analyses at taxonomic level below phylum with differential abundance within groups were made using the Basic Local Alignment Search Tool (BLAST) [21]. Heat maps were generated using MetaboAnalyst 5.0 [22].

### 2.3. Acetate measurements

Quantification of the SCFA acetate was assessed in caecum samples by using a colorimetric assay kit (Sigma, MAK086), according to the manufacturer's specifications. In this test, acetate concentration is determined by a coupled enzyme assay, which results in a colorimetric (450 nm) product proportional to the acetate present.

### 2.4. Data analysis

Food intake was analyzed with two-way ANOVA followed by Tukey's post hoc tests using GraphPad Prism (v9) for Windows (GraphPad Software, La Jolla, CA, USA, www.graphpad.com). Due to munching behavior unrelated to the treatment (it was detected in a random fashion), values associated with such behavior had to be excluded and repeated-measures analyses were not performed. Fluid intake and body weight data were not affected by this behavior and were analyzed with repeated-measures two-way ANOVA with Tukey's post hoc comparisons. The indices for microbiota α-diversity were obtained using PAST software (v3.20; free software for scientific data analysis). The results for α-diversity and ASV counts were analyzed statistically with a two-way ANOVA and subsequent Tukey's post hoc comparisons, using Prism. The indices for β-diversity were calculated and plotted in 3D using R, and statistical analyses were carried out using PAST. The results were analyzed using a two-way NPMANOVA, and post hoc comparisons were made using one-way NPMANOVAs. Taxonomic distributions at phylum and lower taxonomic levels, as well as data from acetate levels in caecum were analyzed in Prism with a two-way ANOVA and subsequent Tukey's multiple comparison tests.

## 3. Results

### 3.1. Effect of Abx on food/fluids intake and body weight in a GWI model fed with HF

There were significant main effects of treatment ($F_{7,402} = 67.17$, $p < 0.0001$) and time ($F_{8,402} = 16.64$, $p < 0.0001$), and a significant treatment X time interaction ($F_{56,402} = 2.38$, $p < 0.0001$) on food intake (Fig. 1A). In the absence of Abx, HF produced a decrease in food intake compared to ND in both controls and GWI-treated mice ($p < 0.0001$ Tukey's test for both pairwise comparisons). While the administration of Abx still resulted in a significant decrease in food intake by HF in the controls ($p < 0.001$), these effects were not present in the GWI mice. For fluids intake, there were significant main effects of treatment ($F_{7,42} = 150$, $p < 0.0001$), time ($F_{9,378} = 123.9$, $p < 0.0001$), subjects matching ($F_{42,378} = 14.61$, $p < 0.0001$), and a significant treatment X time interaction ($F_{63,378} = 13.55$, $p < 0.0001$) (Fig. 1B). Feeding mice a HF reduced the amount of fluids taken by control and GWI-treated mice compared to mice on ND, regardless whether they received Abx or not ($p < 0.0001$ for both groups comparisons with and without Abx). In addition, both control and GWI groups receiving ND +Abx had a significantly lower intake compared to their corresponding group on ND −Abx ($p < 0.0001$ for both controls and GWI). Similarly, controls and GWI on ND +Abx had a significantly higher fluids intake than any of their analogs on HF with or without Abx ($p < 0.01$ for all comparisons). Lastly, there were significant main effects of treatment ($F_{7,42} = 10.09$, $p < 0.0001$), time ($F_{8,336} = 425.3$, $p < 0.0001$), subjects matching ($F_{42,336} = 84.66$, $p < 0.0001$), and a significant treatment X time interaction ($F_{56,336} = 22.22$, $p < 0.0001$), for body weight (Fig. 1C). Control and GWI subjects receiving HF in the absence of Abx exhibited an increase in body weight compared to their corresponding groups receiving ND ($p < 0.01$ for Con_ND −Abx vs Con_HF −Abx and $p < 0.001$ for GWI_ND −Abx vs GWI_HF −Abx). Abx administration maintained this body weight increase in controls fed with HF versus ND ($p < 0.001$) but this diet effect disappeared for the GWI group.

### 3.2. Effects of Abx on the gut microbiome alterations induced by GWI and HF

The number of sequences exceeded an average of 9562 per group. The ASV counts ± standard error for each group were the following: 261 ± 29.6 for Con_ND −Abx, 11.7 ± 2.3 for Con_ND +Abx, 205.8 ± 16.6 for Con_HF −Abx, 10.3 ± 5.3 for Con_HF +Abx, 206.4 ± 23.1 for GWI_ND −Abx, 7.8 ± 3.5 for GWI_ND +Abx, 196.3 ± 12.5 for GWI_HF −Abx, and 8.7 ± 3.1 for GWI_HF + Abx. As expected, Abx administration significantly reduced the number of sequences for the Con_ND, Con_HF, GWI_ND and GWI_HF when compared to their analog group without Abx ($p < 0.0001$ for each pairwise comparison, Tukey's tests). In light of this, and to ensure that any observed differences in microbial diversity among treatment groups were not due to differential sequence depth, we subsampled each sample to 6831 sequences, which was the lowest number of sequences obtained from any of the samples included in α-diversity analyses. Two-way ANOVA analyses of microbial α-diversity revealed significant main effects of GWI + diet ($F_{1,36} = 4242$, $p < 0.0001$), Abx ($F_{3,36} = 17.73$, $p < 0.0001$) and the interaction between GWI + diet and Abx ($F_{3,36} = 15.01$, $p < 0.0001$) for bacterial richness (Chao-1 index, Fig. 2A). Abx administration drastically reduced the richness for Con_ND, Con_HF, GWI_ND and GWI_HF when compared to their analog group without Abx ($p < 0.0001$ for each pairwise comparison, Tukey's tests). In the absence of Abx, HF significantly reduced the richness in the bacterial communities of Con ($p < 0.0001$) and GWI ($p < 0.01$) when compared to their corresponding ND group, and treatment with GWI agents produced a richness decrease in the ND group versus the control ($p < 0.001$).

Analyses of bacterial profile heterogeneity and evenness showed a significant main effect of Abx ($F_{3,39} = 14.21$, $p < 0.0001$ for the Shannon index, Fig. 2B, and $F_{3,40} = 6.1$, $p < 0.01$ for the Simpson 1-D index, Fig. 2C), of GWI + diet ($F_{1,39} = 3601$, $p < 0.0001$ for the Shannon index, and $F_{1,40} = 266.9$, $p < 0.0001$ for the Simpson 1-D index) and the interaction between GWI + diet and Abx ($F_{3,39} = 6.92$, $p < 0.001$ for the Shannon index and $F_{3,40} = 5.45$, $p < 0.01$ for the Simpson 1-D index). As was seen for the Chao-1 index, α-diversity measured with the Shannon and Simpson indexes was also reduced by Abx administration for all groups compared to their analog without Abx ($p < 0.0001$ for all, Tukey's test). Interestingly, while HF in the presence of Abx caused a reduction of the microbial heterogeneity and evenness in controls ($p < 0001$ for Shannon and $p < 0.05$ for Simpson 1-D, Tukey's tests), this diet in combination with Abx was associated with diversity increases in the GWI group ($p < 001$ for Shannon and $p < 0.01$ for Simpson 1-D, Tukey's tests).

Two-way NPMANOVA analyses of β-diversity revealed main effects of GWI + diet ($F_{1,48} = 25.5$, $p < 0.0001$), Abx ($F_{1,48} = 45.92$, $p < 0.0001$) and their interaction ($F_{1,48} = 21.85$, $p < 0.0001$). The Jaccard index (Fig. 3A), which reflects bacterial community composition showed that the ASV profiles of groups clustered by GWI treatment, diet regimen and Abx (all post hoc comparisons among groups were statistically significant at $p < 0.05$). The Bray-Curtis index (Fig. 3B), which indicates the structure of the microbial community showed a similar ASV clustering by the same factor with all group comparisons reaching statistical significance ($p < 0.01$) but one: Con_ND +Abx vs GWI_ND +Abx. This indicates that Abx administration made the differences between Con and GWI-treated mice on ND disappear.

Analyses at the level of bacterial phyla showed that the main effects of phylum ($F_{8,369} = 266.4$, $p < 0.0001$) and the interaction between treatment X phylum ($F_{56,369} = 42.45$, $p < 0.0001$) were significant, whereas the effects of treatment alone were not (Fig. 4A). Abx were effective in decreasing the relative abundance of almost the entire set of the most prominent phyla in the gut with the exception of Proteobacteria. However, only Firmicutes, Bacteroidetes and Proteobacteria reached statistical significance. Without Abx, no differences were found by diet regimen or GWI treatment for Proteobacteria. However, in the presence of Abx, the abundance of Proteobacteria was lower in HF

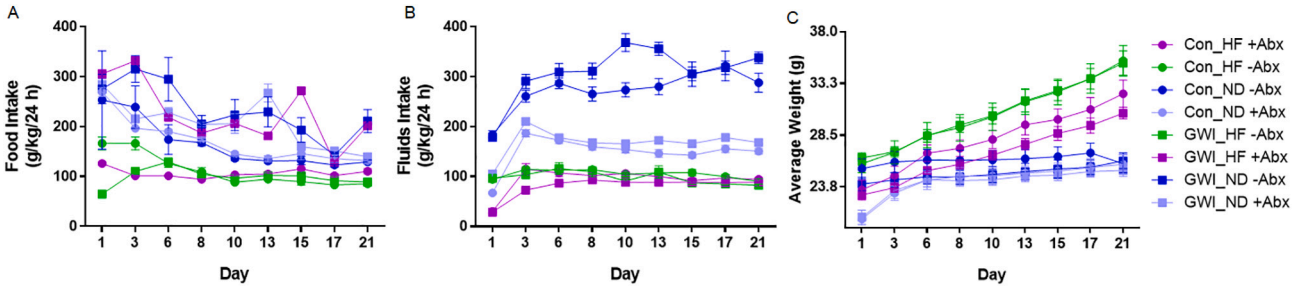


**Fig. 1.** Effect of diet on food intake (A), fluids intake (B), and average body weight (C). Mice were treated with GWI agents or Con (control) and then fed a normal diet (ND) or a high fat diet (HF), concomitantly with antibiotics (+Abx) or without antibiotics (Abx) for 21 days. Food and fluids intake measures were calculated based on food or fluids consumption (g), mouse body weight (kg) for a 24 h period and reported as g/kg/24 h. Results are mean ± SEM, $N = 6–7$.

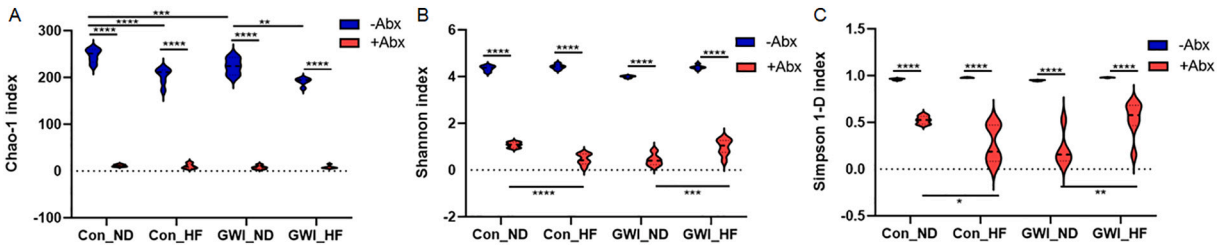


**Fig. 2.** Violin plots of the microbial α-diversity indexes Chao-1 (A), Shannon (B), and inverse Simpson (C) in mice treated with GWI agents or Con (control) and then fed a NORMAL diet (ND) or a high fat diet (HF), concomitantly with antibiotics (+Abx) or without antibiotics (−Abx) for 21 days. Values are mean ± SEM. Symbols represent significance levels for the indicated post hoc comparisons as p<: *0.5, **0.01, ***0.001, and ****0.0001.

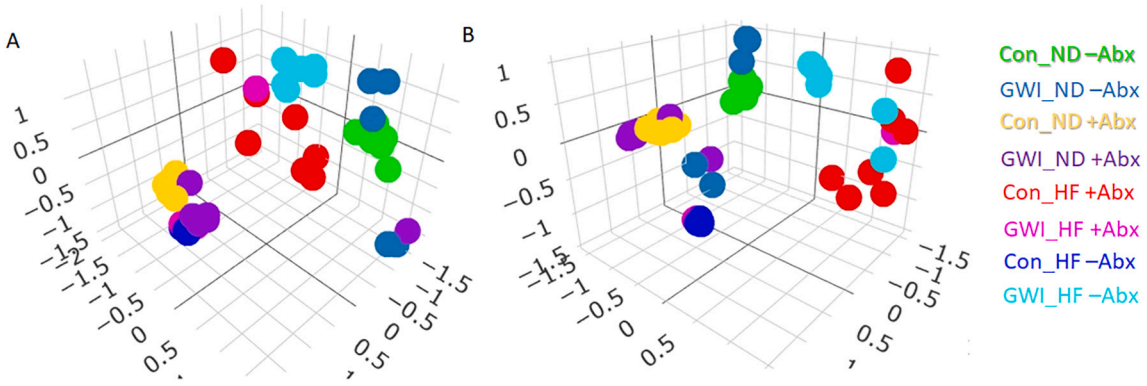


**Fig. 3.** 3D Non-metric multidimensional scaling analyses of the microbial β-diversity indices Jaccard (A) and Bray-Curtis (B) in mice treated with GWI agents or Con (control) and then fed a normal diet (ND) or a high fat diet (HF), concomitantly with antibiotics (+Abx) or without antibiotics (−Abx) for 21 days. $N = 5–7$.

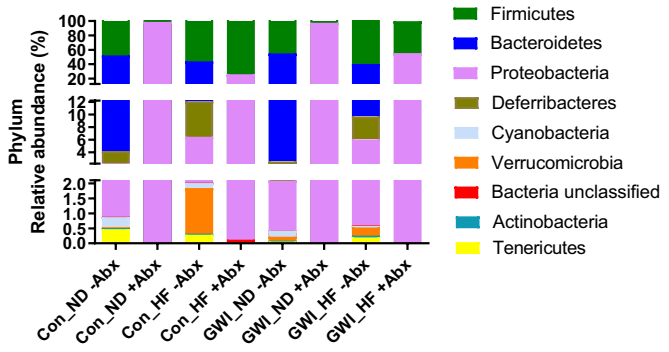


**Fig. 4.** Percent relative abundance of the 8 most prominent bacterial phyla in mice treated with GWI agents or Con (control) and then fed a normal diet (ND) or a high fat diet (HF), concomitantly with antibiotics (+Abx) and without antibiotics (−Abx) for 21 days. $N = 6–7$.

groups compared to their ND analog for both controls and GWI-treated mice ($p < 0.0001$ for both pairwise comparisons).

For ND groups on Abx, the differences between controls and GWI were not significant, whereas for HF with Abx, controls had a lower relative abundance of Proteobacteria compared to GWI-treated mice ($p < 0.0001$). In controls and GWI group without Abx, the relative abundance of Bacteroidetes was higher when receiving ND compared to HF ($p < 0.01$ for controls, and $p < 0.0001$ for GWI). No differences were found between controls and GWI-treated mice when receiving the same diet regimen, whether that was ND or HF. These patterns on Bacteroidetes relative abundance changed in the presence of Abx, when the diet regimen had no effect on either controls or GWI-treated animals. In addition, Abx administration resulted in no differences between controls and GWI within the same diet regimen. In the absence of Abx, diet regimen had no effects on the relative abundance of the Firmicutes phylum in controls, whereas in GWI subjects, HF produced a decrease in this phylum compared to its ND analog ($p < 0.05$). Comparisons between control and GWI groups on the same diet without Abx did not show any significant differences. This indicates that in the absence of Abx, GWI treatment did not cause any effects on the relative abundance of Firmicutes. However, in the presence of Abx HF increased the abundance of Firmicutes in both controls and GWI ($p < 0.0001$ for both pairwise tests) compared to their respective ND groups. GWI treatment did not have any effect on Firmicutes abundance when mice received ND, but it decreased when receiving HF ($p < 0.0001$, Tukey's test).

Analyses of gut bacteria at taxonomic levels below phylum (Fig. 5), showed significant effects for 6 members of Proteobacteria (*Ochrobactrum*, *Klebsiella*, Enterobacteriaceae, Burkholderiaceae, Desulfovibrionaceae, and Rhodospirillales), and a member of Firmicutes (*Lactococcus*). The main effects of GWI treatment ($F_{1,35} = 10.72$, $p = 0.002$ for *Ochrobactrum*; $F_{3,41} = 33.91$, $p < 0.0001$ for *Klebsiella*; $F_{3,34} = 23.06$, $p < 0.0001$ for Enterobacteriaceae; $F_{3,32} = 30.59$, $p < 0.0001$ for Burkholderiaceae; $F_{3,41} = 71.23$, $p < 0.0001$ for *Desulfovibrionaceae*; $F_{3,35} = 10.64$, $p < 0.0001$ for Rhodospirillales; and $F_{3,40} = 41.7$, $p < 0.0001$ for *Lactococcus*), Abx ($F1_{,34} = 12.69$, $p = 0.01$ for *Ochrobactrum*; $F_{1,41} = 178.1$, $p < 0.0001$ for *Klebsiella*; $F_{1,34} = 127$, $p < 0.0001$ for Enterobacteriaceae; $F_{1,32} = 89.62$, $p < 0.0001$ for Burkholderiaceae; $F_{1,41} = 344.2$, $p < 0.0001$ for Desulfovibrionaceae; $F_{1,35} = 129.3$, $p < 0.0001$ for Rhodospirillales; and $F_{1,40} = 68.85$, $p < 0.0001$ for *Lactococcus*) and GWI treatment X Abx interaction ($F_{3,34} = 3.1$, $p < 0.05$ for *Ochrobactrum*; $F_{3,41} = 33.9$, $p < 0.0001$ for *Klebsiella*; $F_{3,34} = 26.14$, $p < 0.0001$ for Enterobacteriaceae; $F_{3,32} = 33.93$, $p < 0.0001$ for Burkholderiaceae; $F_{3,41} = 71.2$, $p < 0.0001$ for Desulfovibrionaceae; $F_{3,35} = 10.6$, $p < 0.0001$ for Rhodospirillales; and $F_{3,40} = 29.18$, $p < 0.0001$ for *Lactococcus*) were all significant. Although and increase in the abundance of the genus *Ochrobactrum* (Fig. 5A) by HF did not reach statistical significance in controls treated with Abx, this increase was significant in Con_HF +Abx for Burkholderiaceae ($p < 0.0001$, Fig. 5E), and *Lactococcus* ($p < 0.0001$, Fig. 5B) compared to Con_ND +Abx. However, the

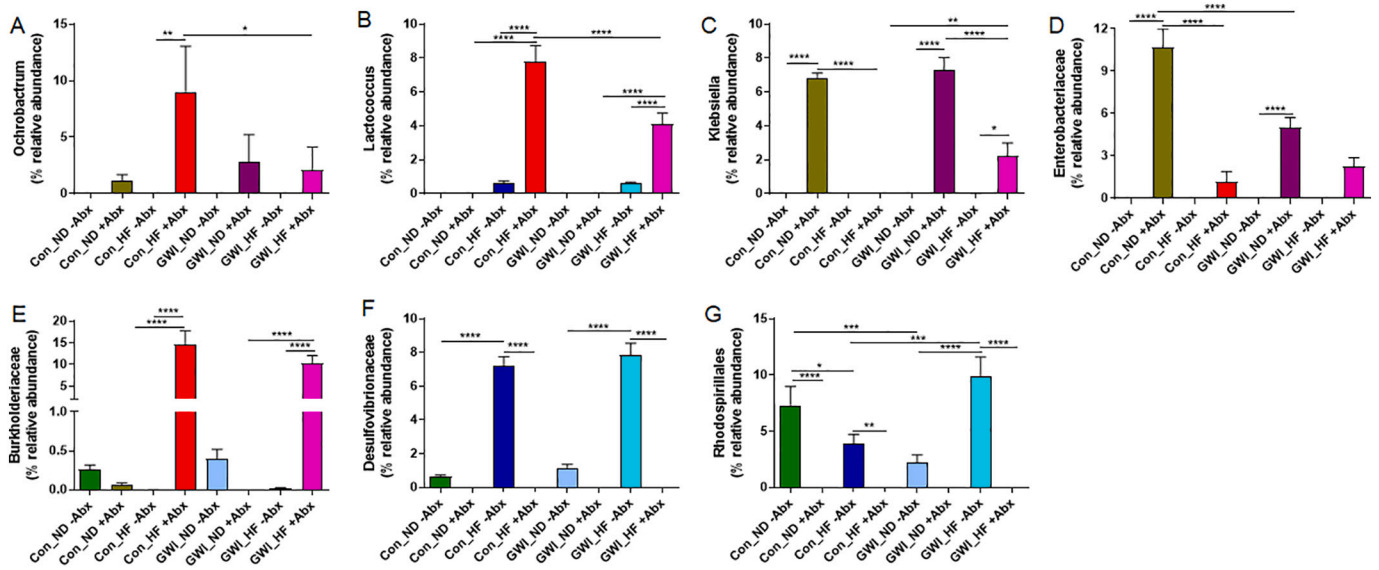


**Fig. 5.** Relative abundance of taxa below the level of phylum in treatment and diet groups. Results are presented as % relative abundance for each taxon. Con = control; GWI = PER + PB; ND = normal diet; HF = high fat diet; −Abx = without antibiotics; +Abx = with antibiotics. Symbols represent significance levels for the indicated post hoc comparisons as p<: *0.05, **0.01, ***0.001; ****0.0001.

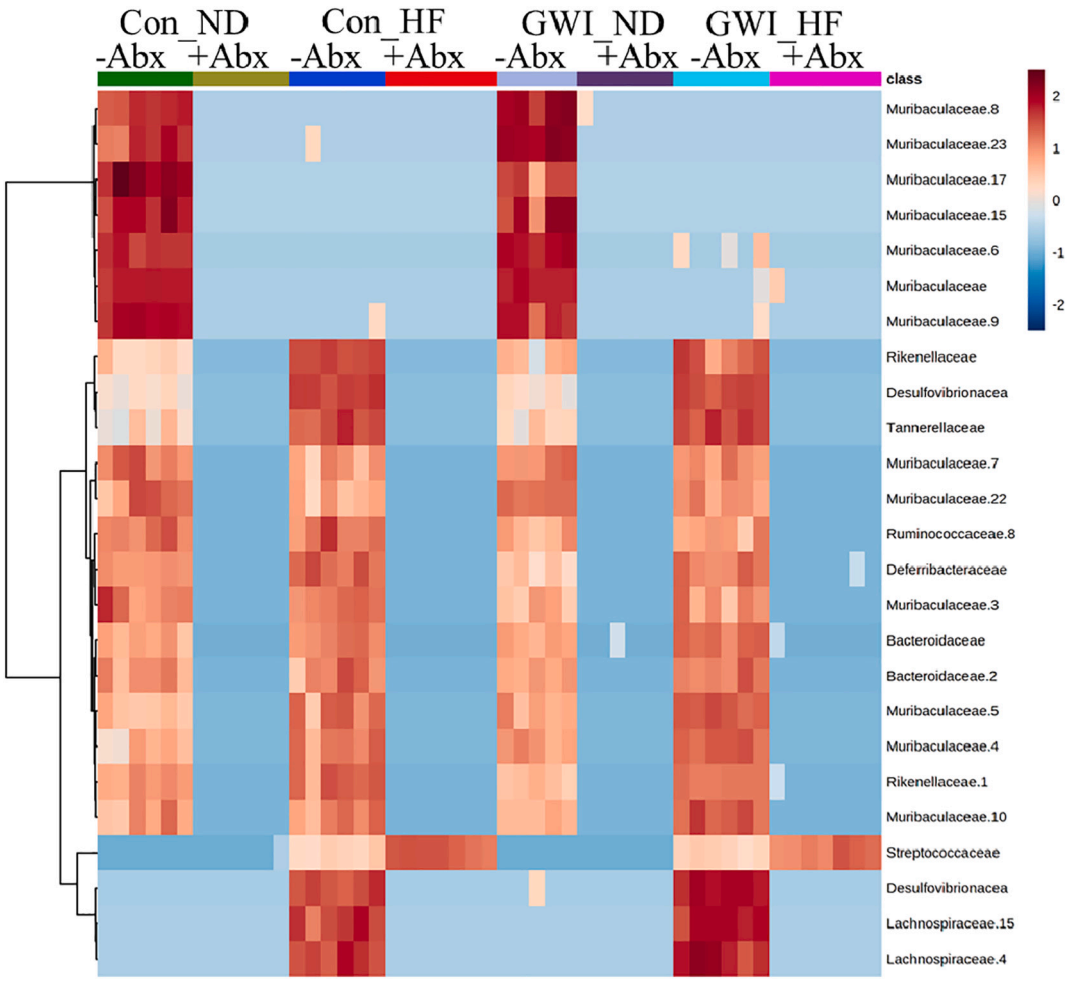


**Fig. 6.** Heat map illustrating patterns in relative abundance of ASVs among the treatment groups. All subjects in each group are arrayed in columns and bacterial taxonomies are indicated in rows. Con = control; GWI = PER + PB; ND = normal diet; HF═HF diet; −Abx = without antibiotics; +Abx = with antibiotics. Clustering along the y-axis was done using the Ward algorithm. *N* = 6–7.

opposite effect of HF was observed in controls exposed to Abx, where the abundance of the genus *Klebsiella* ($p < 0.0001$, Fig. 5C) and the Enterobacteriaceae family ($p < 0.0001$, Fig. 5D) were significantly decreased. Similar patterns of increased abundance of Burkholderiaceae ($p < 0.0001$) and *Lactococcus* ($p < 0.0001$) were observed after HF in GWI-treated mice exposed to Abx compared to their corresponding group fed with ND. The administration of Abx to controls and GWI mice did not cause any changes in Desulfovibrionaceae (Fig. 5F) or Rhodospirillales (Fig. 5G) regardless of the diet. Increases in Klebsiella and Enterobacteriaceae were observed in both controls ($p < 0.001$ for both taxa) and GWI ($p < 0.0001$ for both taxa) mice fed with ND in the presence of Abx. While the abundance of *Ochrobactrum* and *Lactococcus* was significantly decreased by HF in GWI compared to controls in the presence of Abx ($p < 0.05$ for both taxa), the relative abundance of Klebsiella was significantly increased ($p < 0.01$).

In the absence of Abx, HF caused an increase in Desulfovibrionaceae in both controls and GWI-treated mice compared to their analog groups fed with ND ($p < 0.0001$ for both). Comparisons between Con_HF −Abx and Con_HF +Abx in controls and GWI-treated subjects show significant decreases for Desulfovibrionaceae and Rhodospirillales ($p < 0.01$). Interestingly, the effect of HF in controls without Abx was associated with decreases in Rhodospirillales ($p < 0.05$), whereas in the GWI group, this taxon was increased after HF ($p < 0.0001$). Furthermore, treatment with GWI agents produced a decrease compared to controls when both groups received ND in the absence of Abx ($p < 0.0001$).

These treatment-driven differences in taxa composition can be visualized in a heat map (Fig. 6), where the presence of Abx is associated with an overall lower abundance of bacteria (dominated by blue intensities) compared to the groups without Abx (dominated by orange-red intensities). The bottom section of this heat map shows that members of Streptococcaceae are the only ones which are increased in HF +Abx groups.

LEfSe analyses identified specific taxa associated with 3 control groups with categories down to bacterial order level (Fig. 7). Members of the Clostridia class and the Clostridiales order were more abundant in Con_ND −Abx, whereas Gammaproteobacteria were representative of Con_ND +Abx, and Bacilli members were representative of the Con_HF +Abx group.

The sequences of ASVs with differential abundance within groups from the analyses at taxonomic level below phylum were searched in BLAST and 6 classifications were made at the species level (100% genetic identity match; Fig. 8). In the presence of Abx, *Akkermansia muciniphila* was more abundant in HF groups regardless of being a control or GWI-treated ($p < 0.0001$ vs their respective ND −Abx analog group; Fig. 8A), but administration of Abx caused a larger expansion of *A. muciniphila* in both controls and GWI ($p < 0.001$). While the abundance of *Erwinia persicina* was increased in both Con_ND +Abx and GWI_ND +Abx compared to their analog groups without Abx, only controls reached statistical significance ($p < 0.0001$; Fig. 8B). In the presence of Abx, HF was associated with a decrease in *E. persicina* in controls ($p < 0.0001$; Fig. 8C) but had no effect in GWI-treated mice. Although the species *Klebsiella grimontii* was only present in Con_ND +Abx and GWI_ND +Abx, when compared to their corresponding analog without Abx or with HF, only the GWI group was significantly increased ($p < 0.001$ vs GWI_ND −Abx and vs GWI_HF +Abx). In addition, the abundance of *K. grimontii* was higher in GWI_ND compared to Con_ND in the presence of Abx ($p < 0.0001$). The combination of HF with Abx whether in Con or GWI lead to increases in *Burkholderia multivorans* ($p < 0.0001$ for all; Fig. 8D), as this bacterium was not present with either HF or Abx alone in either group. Moreover, there was a significant increase in Con_HF +Abx when compared to GWI_HF +Abx ($p < 0.05$). Increases in *Kalamiella piersonii* were exclusively present in Con_ND +Abx and

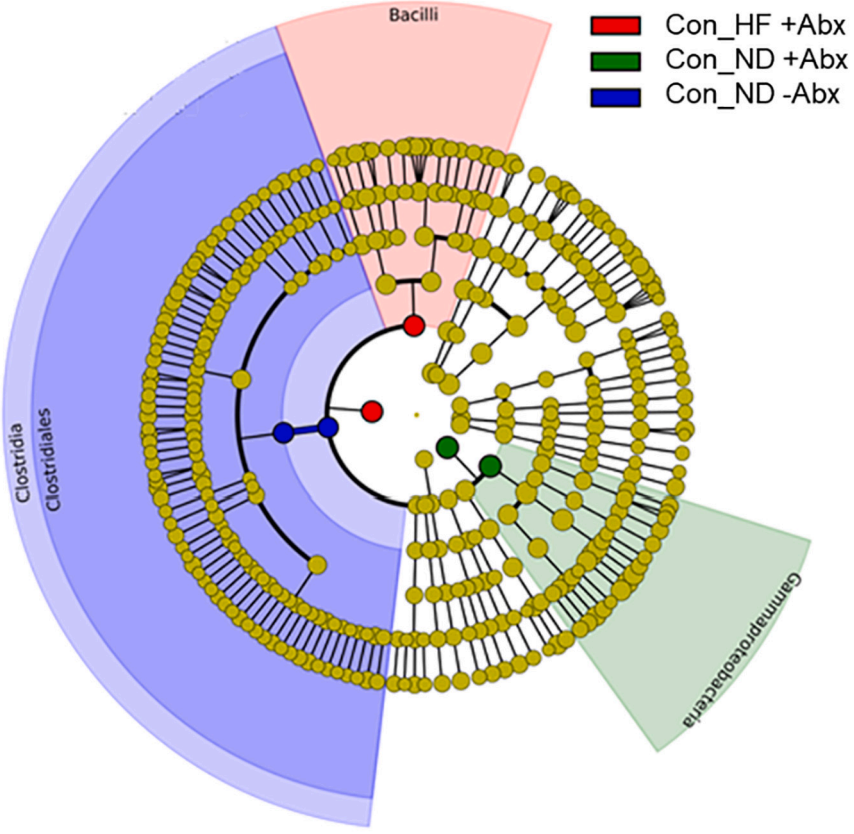


**Fig. 7.** Bacterial taxa that were differentially abundant according to LEfSe analysis. Results are displayed as a cladogram where taxa values in each treatment group are highlighted by small circles and by shading. All groups shown are statistically different compared to each other (LDA > 3.6). Control (Con), High fat diet (HF), with (+Abx) and without (−Abx) antibiotics.

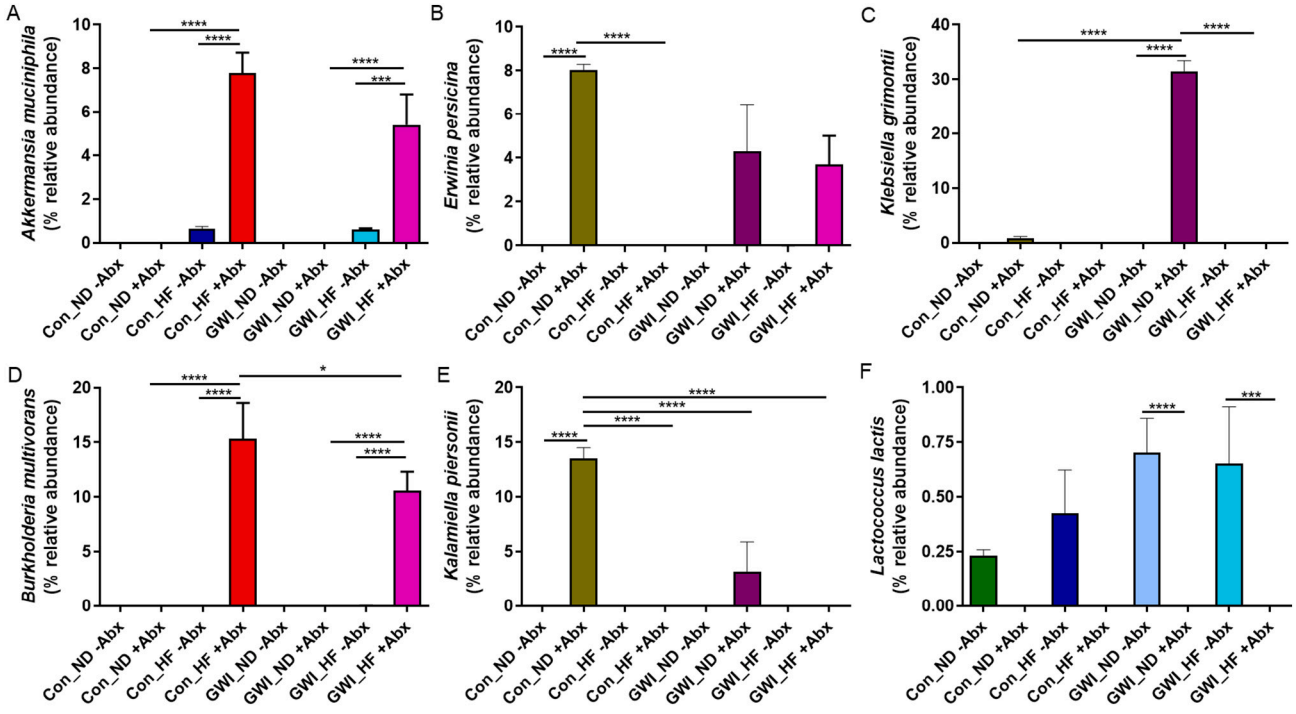


**Fig. 8.** Relative abundance of bacterial species in treatment and diet groups. Results are presented as % relative abundance for each taxon. Con = control; GWI = PER + PB; ND = normal diet; HF = high fat diet; −Abx = without antibiotics; +Abx = with antibiotics. Symbols represent significance levels for the indicated post hoc comparisons as p<: *0.05, ***0.001; ****0.0001.

GWI_ND +Abx (Fig. 8E) but they were only significant for the control group when compared to its control analogs without Abx ($p < 0.0001$) or with HF ($p < 0.0001$), and to GWI_ND +Abx and GWI_HF +Abx ($p < 0.0001$ for both). Lastly, the presence of *Lactococcus lactis* was observed in all treatment groups without Abx (Fig. 8F), but when comparing to their corresponding group in either controls or GWI without Abx, the differences were significant only for the GWI groups ($p < 0.0001$ vs both ND and HF).

### 3.3. Effects of Abx on acetate levels in the caecum of animals treated with GWI agents and fed with HF

Two-way ANOVA analyses revealed significant effects of GWI + diet ($F_{3,23} = 6.74$, $p < 0.01$), Abx ($F_{1,23} = 62.35$, $p < 0.0001$) and their interaction ($F_{3,23} = 19.31$, $p < 0.0001$) on the levels of the SCFA acetate (Fig. 9). Post hoc tests showed that in the absence of Abx, HF decreased the amount of acetate compared to controls ($p < 0.05$ for Con_ND −Abx vs Con_HF −Abx), and treatment with GWI agents in mice receiving a ND also caused an acetate reduction compared to its corresponding Con_ND group ($p < 0.01$). In controls fed with HF, the acetate levels were lower in the group without Abx compared to the one receiving Abx ($p < 0.001$). Treatment with GWI agents is associated with significant increases in acetate levels when combined with either HF ($p < 0.0001$ for GWI_ND −Abx vs GWI_HF −Abx) or Abx ($p < 0.001$ for GWI_ND −Abx vs GWI_ND +Abx).

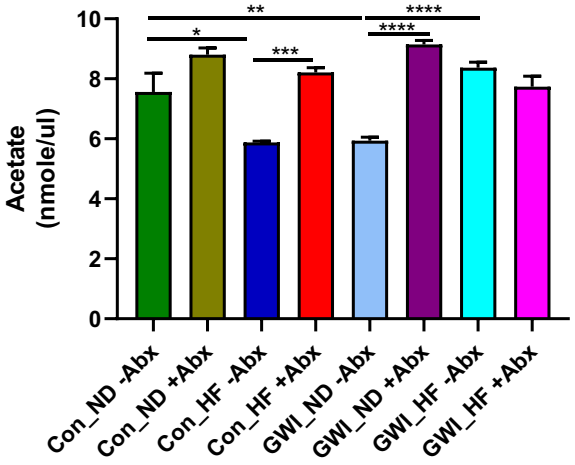


**Fig. 9.** Levels of acetate in caecum of mice treated with GWI agents or Con (control) and then fed a normal diet (ND) or high fat diet (HF), concomitantly with antibiotics (+Abx) or without antibiotics (−Abx) for 21 days. Results are mean ± SEM, $N = 4–5$. Symbols represent significance levels for the indicated post hoc comparisons as p <: *0.05, **0.01, ***0.001, ****0.0001.

## 4. Discussion

Results show that while treatment with GWI agents does not change the HF-driven increase in body weight, administration of Abx was effective in counteracting this increase only in GWI subjects as controls showed a tendency towards a body weight decrease with Abx but this was not statistically significant (see Fig. 1). This offsetting effect of Abx on the body weight increase by HF in GWI subjects was unexpected in light of the significant increase in food intake observed in the GWI group receiving HF +Abx. While this finding is counterintuitive and requires future investigation, the fact that it only took place in the presence of Abx pinpoints the gut microbiota as a mediator in the body weight effects of HF in GWI. A similar capacity of Abx to decrease the body weight gain induced by HF was documented in C57Bl/6 mice [23] but no reports on the effects of Abx on body weight of GWI Veterans was found.

While the use of Abx was effective in reducing the ASV counts to less than 5% of the groups without Abx (see ASV counts above), this drop favored the proliferation of certain bacterial taxa. In this sense, a previous study in mice using Abx and a sequencing approach to assess gut bacteria alterations yielded a significant reduction of about 50% of

bacterial species [24]. When quantifying colony-forming units (CFU) from fecal cultures, the bacterial decreases after Abx exposure are more pronounced, ranging from 1 million-fold to non-detectable [25,26]. These discrepancies are explained by the CFU method being restricted to the measurement of culturable gut microbes. Strikingly, a large number of published reports employing the broad-spectrum Abx as an approach to deplete the gut microbiota, do not document any data on the extent of such depletion [27–30].

Our previously reported findings that treatment with GWI agents as well as with HF caused a significant reduction in microbial richness [2] were corroborated, and the present study found that exposure to Abx accentuated even more these decreases in α-diversity. Such reduction in microbial α-diversity measures has been consistently reported as a result of HF [2,31,32] and Abx exposure [24,25,33].

Besides altering α-diversity, treatment with GWI and HF changed the composition and structure of the microbial gut community in a way that was not prevented by administration of Abx (see Fig. 3). More specifically, it was found that GWI and HF each had an effect on the relative abundance of the most prevalent bacterial phyla (Fig. 4A). HF reduced the abundance of Bacteroidetes while increasing that of Firmicutes, and this effect was potentiated with the administration of Abx, which caused a complete depletion of Bacteroidetes (Fig. 4B). Beyond their capacity to alter microbial β-diversity [33,34], Abx favored the expansion of Proteobacteria members. These overall findings have been documented [24,34] but the extent of the shifts in each phylum differ, depending on the type of samples used (i.e. adolescent vs adult age, or rat vs mouse samples). Increases in the phylum Proteobacteria have been reported in the aged gut microbiome [35], and in conditions coursing with inflammation such as inflammatory bowel disease and Alzheimer's disease [36,37]. Similar shifts in bacterial phyla favoring a Proteobacteria outgrowth have been reported in studies with broad-spectrum Abx [24,34]. In terms of gut microbiome alterations attributed to GWI, preliminary data from a pilot study of Gulf War (GW) Veterans with and without gastrointestinal symptoms reported increases in Proteobacteria in a subset of individuals with both GWI and gastrointestinal symptoms [9]. Furthermore, this proliferation of Proteobacteria was associated with greater levels of inflammatory cytokines in plasma [9]. A double-blind, placebo-controlled trial in GW Veterans with irritable bowel syndrome (IBS), a common condition in GWI, reported that the use of Abx was not associated with significant improvement in IBS-related symptoms [38]. However, a close analysis of these results called for a more cautionary interpretation as albeit having multiple strengths, this study also had weaknesses associated with an underpowered sample size [39]. The Abx-induced restructuring of the microbial community demonstrates that Abx administration is one of the most pervasive ways to disrupt the gut microbiome, as observed by the capacity of these compounds to induce dysbiosis.

Deeper analyses of bacterial taxa below the phylum level evidenced increases in the genera *Ochrobactrum* and *Lactococcus* produced by the combination of HF with Abx, which were of greater magnitude in controls than in GWI subjects (Fig. 5A and B). This is consistent with reports of *Ochrobactrum* increases in the intestinal mucosa of mice fed with HF [40]. Interestingly, the presence of *Lactococcus* has been consistently documented as an outcome of HF consumption [41], but it was later found that this was the result of dietary contaminants in most commercially available HF (i.e. casein), and that the high levels of bacteria found in casein-containing HF were intact but likely dead cells as they failed to proliferate in culture [41]. While casein was a component of the HF formulation employed in the present study, and this factor could explain the increases in Lactococcus observed in the HF groups, the fact that such increases were further heightened by Abx in both control and GWI groups points to a synergic effect of Abx with HF, and suggests that either *Lactococcus* in the HF groups were not dead and their outgrowth was promoted by Abx in the absence of other competitors, or that baseline levels of *Lactococcus* were not only resistant to Abx but also capable of proliferating in a non-adversarial environment. It is noteworthy that *Lactococcus* is significantly more abundant in controls than in GWI subjects exposed to HF and Abx. Given that *Lactococcus* is considered a beneficial bacterial genus with members that constitute probiotic strains [32], the decreases in this genus in GWI relative to controls could be associated with worse outcomes.

Two taxa of gram-negative bacteria, including the genus Klebsiella and the family Enterobacteriacea were overrepresented not only in the groups treated with Abx in a fashion that seemed diet-independent (Fig. 5C and D). While the abundance of these two bacteria was greater in the groups receiving a ND with Abx, the combination of HF with Abx also led to increases. Exposure to Abx in GWI-treated mice was associated with lower abundance of Enterobacteriacea than in controls. These two taxa belong to the betaproteobacteria and gammaproteobacterial classes respectively, and contain members that cause human disease and are resistant to Abx [42,43]. The multiplication of bacteria capable of causing disease in the control groups receiving Abx points to the adverse effects of this seemingly innocuous intervention. Although the families Burkholderiaceae and Desulfovibrionaceae were significantly altered by HF, neither diet nor treatment with GWI seemed to be determinant factors (Fig. 5E and F). However, the order Rhodospirillales, seemed sensitive to treatment with GWI, diet and Abx exposure. This taxon was only detected in the absence of Abx, and was found less abundant in the GWI group fed with ND compared to HF, while in controls Rhodospirillales was more abundant with ND. This order of bacteria comprises members that produce acetic acid [44] but also some strains capable of utilizing acetate as a growth source [45]. In light of the increased acetate levels observed in the groups exposed to Abx (see Fig. 9), it is likely that the members of Rhodospirillales thriving in the absence of Abx are the strains that produce acetic acid. The lower abundance of this order in GWI-treated mice on ND and the subsequent increase in its analog fed with a HF supports this hypothesis.

The increased abundance of the Clostridia class, and the Clostridiales order particularly in controls fed with ND without Abx (considered the true control in this study), in comparison to the rest of the groups with HF or GWI treatment, indicates that these two interventions alter host's health (see LEfSE cladogram, Fig. 7). Clostridia, a class of 20–30 beneficial bacteria has been identified as a crucial factor for maintenance of gut homeostasis [46] and can prevent mice from becoming obese [47]. Similarly, decreases in Clostridiales has been associated with type 2 diabetes in mouse models [48]. HF in combination with Abx in controls was associated with increases in gram-positive Bacilli, which contain several well-known pathogens. As revealed by LEfSe, Abx also disrupted gut homeostasis and led to a proliferation of Gammaproteobacteria not only in controls, but also in subjects treated with GWI as evidenced by increases in *K. grimontii* and K piersonii (see Fig. 8C and E). *E. persicina* was also increased in the control group by Abx independent of the diet (Fig. 8B). Preliminary data from an infection model with *E. Persicina* suggest that this bacterium causes diarrhea and increases liver inflammation [49]. In addition, *L. lactis* was overrepresented in the GWI group with and without HF, which is consistent with data from GWI mouse models showing increases in L. *lactis* in GWI fed with a Western-style diet (WD) [5], that is rich is fat and carbohydrates. This study also found increases in *A. muciniphila* in the group treated with GWI + WD, which we observed increased with HF, regardless of treatment (Fig. 8A). While *A. muciniphila*, a mucin-degrading bacterium is suggested to play a protective role in the gut and in reversing the metabolic alterations induced by HF, mucin degradation could compromise the integrity of the entire mucus barrier, which is vital for health maintenance [50]. Thus, a HF-driven increase in *A. muciniphila* could also be interpreted as a possible mechanism for this detrimental diet to disrupt the gastrointestinal tract. Moreover, *B. multivorans* was characteristic of the groups fed with HF and exposed to Abx (see Fig. 8D). The genus *Burkholderia* comprises metabolically diverse bacteria that are known to thrive in adversarial environments. This is the case of *B. multivorans*, which is an opportunistic pathogen displaying significant Abx resistance [51]. The increased abundance of this microbe in the Abx group could be a result

of its capacity to survive in the presence of these compounds.

Treatment with GWI produced a clear reduction in the acetate levels compared to controls but the effects of HF on acetate depended on the treatment (see Fig. 9). In controls without Abx, HF significantly reduced the levels of acetate whereas in the GWI group without Abx HF caused an increase. The reports indicating that HF is associated with decreases in SCFAs, including acetate [52] are consistent with the effects observed in controls. Furthermore, the acetate concentration was reduced in feces of overweight and obese humans compared to healthy controls [15]. In the case of GWI_HF −Abx group, the dysbiosis favoring an increase in acetate-producing bacteria such as *Ochrobactrum* [53], could explain the increases in this SCFA. Exposure to Abx tended to increase the levels acetate in a diet-independent manner. The only exception was the GWI_HF +Abx group, which was not significantly different from its analog without Abx. These results stand in contrast to a study showing that the production of the SCFAs acetate, propionate and butyrate by the colonic microbiota are significantly reduced by Abx [33]. These overall increases in acetate in the groups exposed to Abx could also be enhanced by an expansion of potential acetate consumers in groups without Abx, such as Rhodospirillales.

## 5. Conclusion

In light of these results, it can be concluded that the perturbations caused by GWI and HF were only partially prevented by Abx. Feeding a HF decreased both food intake and body weight gain in the GWI groups exposed to Abx. However, these offsetting effects of Abx were not translated into restitution of microbial diversity or normalization of beneficial bacterial byproducts such as acetate. Furthermore, Abx themselves caused a remodeling of the gut microbiome that was associated with decreases in α-diversity, changes in the composition and structure of the microbial community characterized by a large expansion of Proteobacteria members, and acetate levels. While Abx were very effective in reducing the two dominant gut microbiome phyla (i.e. Firmicutes and Bacteroidetes), they favored the proliferation of potential pathobionts. Although the detrimental effects of treatment with GWI on the gut microbiome and their aggravation by HF were tangible, the use of Abx may not represent a dependable approach to deplete the gut microbiome and its advantages as a pseudo germ-free model warrant further investigation.

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## CRediT authorship contribution statement

**Mariana Angoa-Pérez:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration. **Branislava Zagorac:** Investigation, Data curation. **Dina M. Francescutti:** Investigation, Formal analysis. **Kevin R. Theis:** Formal analysis, Data curation, Writing – review & editing. **Donald M. Kuhn:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influenced the work reported in this paper.

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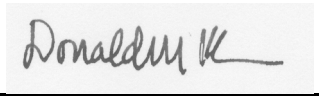
CURRICULUM VITAE

**Donald M. Kuhn, Ph.D.**



Donald M. Kuhn, Ph.D.

Date of Preparation: 08/09/2021


| | |
|---|---|
| Office Address: | Department of Psychiatry and Behavioral Neurosciences<br>Wayne State University School of Medicine<br>John D. Dingell VA Medical Center<br>4646 John R<br>Research Service (11R), Room B4281<br>Detroit, MI USA 48201 |
| Office Telephone: | 313-576-4457 |
| Facsimile: | 313-576-1112 |
| Laboratory: | 313-576-4520 |
| e-mail: | donald.kuhn@wayne.edu<br>donald.kuhn@va.gov |
| Home Address: | 3802 Crestlake Drive<br>Bloomfield Hills, MI 48304 |
| Home Telephone: | 248-642-1514 |
| Cellular: | 248-496-4905 |


## EDUCATION

Baccalaureate: Presbyterian College, Clinton, South Carolina, B.S. degree 1972

Graduate: University of South Carolina, Columbia, S.C., Ph.D. degree 1976

## TRAINING

Aug 1972-Aug 1976 Graduate research and teaching assistant, Department of Psychology, University of South Carolina, Columbia, S.C. 29208

May 1974-Aug 1974 Graduate research assistant, Department of Biochemistry, Medical University of South Carolina, Charleston, S.C. 29301

Sep 1974-Jun 1976 Graduate research assistant, Ensor Research Laboratory, William S. Hall Psychiatric Institute, Columbia, S.C. 29202

Sep 1976-Apr 1977 Postdoctoral Fellow, Program in Neuroscience, Department of Psychology, Princeton University, Princeton, N.J. 08540

May 1977-May 1978 NIH Postdoctoral Fellow, Section on Biochemical Pharmacology, Hypertension-Endocrine Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, M.D. 20205

**FACULTY and ACADEMIC APPOINTMENTS**

Sep 1981-Mar 1986 Consultant in Research
Department of Pharmacology
The George Washington University Medical Center
Washington, D.C.

Apr 1986-Dec 1991 Associate Professor
Department of Psychiatry
Wayne State University School of Medicine
Detroit, Michigan

Dec 1991-present Professor, with tenure
Department of Psychiatry and Behavioral Neurosciences
Wayne State University School of Medicine
Detroit, Michigan

Dec 1994-2010 Adjunct Professor and Member
NIEHS Center in Molecular and Cellular Toxicology with Human Applications
Institute for Chemical Toxicology
Wayne State University
Detroit, Michigan

Sep 1998-present Research Career Scientist
John D. Dingell VA Medical Center
Research & Development Service (11R)
Detroit, Michigan

Feb 2017-Feb. 2018 Assistant Chief of Staff (Acting), Research & Development Service
John D. Dingell VA Medical Center
Detroit, Michigan

Feb 2018-present Deputy Assistant Chief of Staff, Research & Development Service

John D. Dingell VA Medical Center
Detroit, Michigan

Feb 2017-present Research Integrity Officer
John D. Dingell VA Medical Center
Detroit, Michigan

**OTHER PROFESSIONAL APPOINTMENTS**

Jun 1978-Apr 1983 Senior Staff Fellow, Section on Biochemical Pharmacology, Hypertension-Endocrine Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD

Apr 1983-Sep 1985 Pharmacologist, Section on Biochemical Pharmacology, Hypertension-Endocrine Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD

Aug 1985-Mar 1986 Visiting Scientist
J.W. Goethe Universitat
Zoologisches Institut
6000 Frankfurt am Main
Federal Republic of Germany

Sep 1985-Feb 1986 Chief, Section on Biochemical Pharmacology
Hypertension-Endocrine Branch
National Heart, Lung, and Blood Institute
National Institutes of Health
Bethesda, MD

Feb 1986-Nov 1992 Director, Laboratory of Neurochemistry and Director of Research
Lafayette Clinic
Detroit, Michigan

Aug 1993-Jun 1994 Visiting Professor
Department of Molecular Genetics and
Howard Hughes Medical Institute
University of Texas Southwestern Medical Center at Dallas, Texas

**MAJOR PROFESSIONAL SOCIETIES**

- American Society for Neurochemistry (ASN)
- American Society for Pharmacology and Experimental Therapeutics (ASPET)
- International Drug Abuse Research Society (IDARS)
- Society for Neuroscience (SFN)

- Federation of American Societies for Experimental Biology (FASEB)
- National Neurotrauma Society (NNTS)
- International Behavioral Neuroscience Society (IBNS)
- International Society for Neurochemistry (ISN)

**HONORS AND AWARDS**

- National Research Service Award, National Heart Lung and Blood Institute (Sponsor: Dr. Walter Lovenberg), 1976

- Mead-Johnson American College of Neuropsychopharmacology (ACNP) Travel Award, San Diego, California, 1981

- Merck, Sharpe and Dohme Visiting Scholar, Department of Medicine (Host: Dr. J. Chalmers), Flinders Medical Center, Bedford Park, South Australia 5042, 1982

- Vector Laboratories Outstanding Young Investigator Award in Neurochemistry, 1983

- FASEB Travel Award, IUPHAR International Congress of Pharmacology London, England, 1984

- Alexander von Humboldt Fellow, J.W. Goethe University (Host: Prof. Dr. H. Zimmermann), Frankfurt am Main, Federal Republic of Germany, Member of AvH 1985- present

- Wayne State University, Neuroscience Research Award, 1986

- Research Career Scientist, Department of Veterans Affairs, 2006-present

- Research Excellence Award 2012, Wayne State University School of Medicine

- Federal Employee Recognition Award, 2017, Detroit Federal Executive Board

**LICENSES**

- Drug Enforcement Administration Schedule 1 Controlled Substances
- Drug Enforcement Administration Schedule 2, 2N, 3, 3N, 4, and 5 Controlled Substances
- State of Michigan Schedule I Controlled Substance License
- State of Michigan Research Laboratory Controlled Substance License

**SERVICE**

**Wayne State University**

<u>Department of Psychiatry and Behavioral Neurosciences</u>

- Executive Education Committee (EEC) Ex-Officio as Graduate Officer, CCN

- Executive Research Directors Committee (ERDC) Ex-Officio consultant as Graduate Officer, Cellular and Clinical Neurobiology PhD program
- Graduate Officer and Chairman of the Graduate Committee, Cellular and Clinical Neurobiology PhD Program, Department of Psychiatry, Wayne State University, Jan. 1996- Jan. 1998.
- Faculty Promotion and Tenure Committee
  Elected member, Sep 1997 -Sep 2001
  Re-elected Jan 2006- 2010
  Re-elected Mar 2016-present
- Masters of Science in Psychiatry Program
  Ex-Officio member of Program Committee
  Ex-Officio member of Graduate Program
- Protocol Review Committee, Appointed member Oct. 1999 to 2000
- Departmental Leadership Committee, Basic Scientist Representative, Jan. 2012 to present
- Committee to Engage Medical Students and Undergraduates in Departmental Activities (Chair), June 2012-present
- Departmental Research Committee, Nov. 2011-present
- Asselin Award Committee, June 2012-present
- Departmental Publications Committee, July 2012-present
- Departmental New Investigator Research Grants Committee (Co-Chair), Oct. 2012-present
- Departmental Chair's Committee on Funding Innovative Pilot Projects, Dec. 2012-present
- Translational Neuroscience Program (PhD), Steering Committee, Jun. 2014- present
- Translational Neuroscience Program Assessment and Performance, Wayne State University Compliance Assist, Office of the Provost, June 2016-present
- Psychiatry Resident's Summer Seminar Program, Course Director and Lecturer, May 2016-present
- Departmental Faculty Search Committee October 2018- present

<u>School of Medicine</u>

- Wayne State University School of Medicine, Interdisciplinary Biological Sciences PhD Program Executive Committee (Departmental representative)
- Member, IBS Systems Biology Curriculum Subcommittee
- Director, Neurosciences Component of the Systems Biology Course
- Member, School of Medicine Task Force on Graduate Assistantships
- Member (Appointed by Dean of the School of Medicine), Chair Search Committee for the Department of Psychiatry and Behavioral Neurosciences, 2003
- Member (Elected by faculty), Department of Psychiatry & Behavioral Neurosciences Committee, 5-Year Departmental Review, 2007.
- Wayne State University Department Faculty Developmental Liaison Group (Departmental Representative), March 2012-present
- Member (elected) of the Wayne State University School of Medicine Hearing Panel, Office of the Dean, Aug. 2016 – Aug 2017
- Chair, School of Medicine Departmental 5 Year Review, Department of Pathology

<u>University</u>

- Member, OVPR Research Focus Group for Development of School of Medicine Strategic Plan

- Member, Wayne State University Division of Laboratory Animal Resources (DLAR) Advisory Panel, Feb. 2015- present
- Search Committee, Wayne State University, Office of the Vice President for Research, Division of Laboratory Animal Resources Attending Veterinarian Candidate Search Committee, Oct, 2016- Nov. 2017
- Member, Wayne State/VA Joint Committee on Human and Animal Research Activities, Mar. 2018- present

Affiliate Medical Organizations

- Member, John D. Dingell VA Medical Center R&D Committee
  Member, January 2001-January 2002
  Chair, January 2002 to January 2004
  Member, January 2005 to June 2014
  Chair, June 2014 to January 2017
- Member, John D. Dingell VA Medical Center Research Review Committee
  Chair, June 2012 - June 2014
  Member, June 2014 - June 2018
  Chair, August 2018 - present
- Member, John D. Dingell VA Medical Center Search Committee for Assistant Chief of Staff, Research & Development Service, Sep 2016- Jan 2017
- Assistant Chief of Staff, Research & Development Service (Acting), Jan 2017- Feb. 2018
- Deputy Assistant Chief of Staff, Research & Development Service, Mar 2018-present
- Member of the Board, The Metropolitan Detroit Research and Education Foundation (MDREF; VA), May 2017-present
- Member. Clinical Executive Committee, John D. Dingell VA Medical Center, Jan. 2017-Feb. 2018
- Member, Affiliation Partnership Council, John D. Dingell VA Medical Center, Jan. 2017-present

**Scholarly Service**

Grant Review Committees

- Ad hoc reviewer for the Neurosciences Research Review Committee of the National Institutes of Mental Health and for the Behavioral and Neurosciences Review Committee of the National Institutes of Health (1985).

- Ad hoc reviewer for the Program in Neural Mechanisms of Behavior and for the Program for Developmental Neuroscience of the National Science Foundation (1988).

- Ad hoc reviewer for the Drug Abuse Biomedical Research Review Committee Pharmacology II Subcommittee (DABR3), National Institute on Drug Abuse (1992-1995).

- Full member of the Drug Abuse Biomedical Research Review Committee NIDA-C, National Institute on Drug Abuse (1994-1998).

- Ad hoc reviewer for the National Institute on Alcohol Abuse and Alcoholism, Office of Scientific Affairs, Contract Review Unit (1995-1998).

- Ad hoc reviewer of scientific grant applications for the Medical Research Council of Canada and for the Netherlands Organization for Scientific Research, Council for Medical and Health Research (Nov. 1999).

- Ad hoc reviewer for Neurological Sciences and Disorders B (NSD-B), National Institute of Neurological Disorders and Stroke (Aug. 2000- Aug. 2002).

- Full member of Molecular, Developmental, and Cellular Neuroscience-4 (MDCN-4) Study Section, Center for Scientific Review, NIH (Feb. 1998-June 2002).

- Full member, Integrative, Functional, and Cognitive Neuroscience (IFCN-7) Study Section (Feb. 2002- Feb. 2006).

- Full member, American Federation for Aging Research Scientific Board (Dec. 2001-Dec. 2004).

- Reviewer, Alzheimer's Association Grant Review Committee (Mar. 2002-Mar. 2004).

- Ad hoc reviewer, Integrative, Functional, and Cognitive Neuroscience (IFCN-4) Study Section (June 2002- June 2004).

- Full member, Neurobiology-A Merit Review Subcommittee, Department of Veterans Affairs (June 2004- June 2008).

- Ad hoc reviewer, Special Emphasis Panel NIMH ZMH1 BRB-S, Molecular Markers and Mechanisms of HIV-Associated Dementia, National Institute on Mental Health (July 2004).

- Reviewer, Agency for Science, Technology & Research, Biomedical Research Council (Singapore), Extramural Grant Program (June 2004).

- Reviewer, Philip Morris External Research Program (July 2005-Nov. 2007)

- Ad hoc reviewer, Special Emphasis Panel NIMH ZMH1-ERB-Y, ADHD and Long-Term Psychostimulant Therapy (March 2005).

- Ad hoc reviewer, Neurobiology of Motivated Behavior (NMB) Study Section (June 2005- June 2006).

- Ad hoc reviewer, NIMH-ERB-L-04, Silvio Conte Centers for Depression and Anxiety (Feb. 2006).

- Ad hoc reviewer and Committee Chair, MDCN-L 02S, Biophysics and Neuronal Processes 1 (Apr. 2006).

- Full member, Neurobiology of Motivated Behavior (NMB) study section (June 2006-June 2010)

- Ad hoc reviewer, NIMH-ERB-L-03, Silvio Conte Centers for Collaborative Neuroscience Research (Mar 2007)

- Full member and Deputy Chair, ZRG1 MDCN-E, Review of Neuroscience AREA-R15 Grant Applications (Nov. 2011- Nov. 2019; Chair Feb. 2020 - present)

- Ad hoc reviewer, ZRG1 IFCN H 02M, Member conflict reviews (Jan. 2012)

- Full member, Department of Veterans Affairs, RRDB 1, Brain Injury (Dec. 2011-Dec. 2013)

- Ad hoc reviewer, ZRG1 BBBP-J 92 study section (Sep. 2012)

- Ad hoc reviewer, ZDA1 GXM-A (14) 1 study section to review NIDA CEBRA grants (Nov. 2012)

- Ad hoc reviewer, ZDA1 SXC-E (13), NIDA Cutting-Edge Basic Research Awards (CEBRA) grant application online IAR review (Mar. 2013)

- Ad hoc reviewer, ZDA1 MXL-F (08) 1, NIDA EUREKA proposal telephone review (Jul. 2013)

- Ad hoc reviewer, ZDA1 SXC-E (13), NIDA Cutting-Edge Basic Research Awards (CEBRA) grant application online IAR review (Apr. 2015)

- Ad hoc reviewer, Department of Veterans Affairs, RRD6 Aging & Neurodegenerative Diseases Merit Review Panel (Aug 2016-present)

- Ad hoc reviewer, National Science Center, Poland, Panel NZ7- Influence of New Psychoactive Drugs, grant application online review, Oct 2016

- Ad hoc reviewer, ZRG1 IFCN-L (56), NIDA Synthetic Psychoactive Drugs and Strategic Approaches to Counteract Their Deleterious Effects Review Panel, Nov. 2017

-Ad hoc reviewer, Department of Veterans Affairs, RRD8, Career Development Program Panel 1, telephone reviewer, Aug. 2019- present.

- Ad hoc reviewer, Department of Veterans Affairs, RRD7, Research Career Scientist Award Applications, Aug. 2020- present.
- Member, 2019 Gulf War Illness Research Program Review Panel, CDMRP, Oct. 2019 – present.

<u>Service for Peer Reviewed Journals Journal/Editorial Activity</u>

- Editorial Board Membership
    Journal of Neurochemistry (1998-2010)
    Neurochemistry International (1984-1994)
    Pteridines (1988-1995)

- Review of Manuscripts
    Behavioural  Brain Research
    Biological Psychiatry
    Brain Research
    Brain Research Bulletin
    Depression and Anxiety
    Drug and Alcohol Dependence
    European Journal of Pharmacology
    Experimental Neurology
    FASEB Journal
    FEBS Letters
    Free Radical Biology and Medicine
    Journal of Biological Chemistry
    Journal of Pharmacology and Experimental Therapeutics
    Journal of Neurochemistry
    Journal of Neuroinflammation
    Journal of Neurological Sciences
    Journal of Neurotrauma
    Journal of Neuroscience
    Journal of Neuroscience Research
    Molecular Neurobiology
    Molecular Pharmacology
    Neurobiology of Disease
    Neuropsychopharmacology
    Neuroscience
    Neurotoxicology
    Neurotoxicology and Teratology
    Pharmacology, Biochemistry and Behavior
    Psychopharmacology
    Synapse

Other Service

- Councilor, Michigan Society for Neuroscience Chapter, Wayne State Representative, Sep. 2000-  Sep 2002

**TEACHING**

Years at Wayne State University: 30
Years at other universities
- Princeton University: 1 (Postdoctoral Fellow; Dr. B. Jacobs)

- The George Washington University: 6 (Adjunct Faculty while member of NIH Intramural Research Program)
- J.W. Goethe University (Frankfurt, Germany): 1 (Alexander von Humboldt Fellow; Dr. H. Zimmermann)
- University of Texas, Southwestern Medical Center at Dallas: 1 (Sabbatical; Dr. T. Sudhof)

Teaching at Wayne State (Graduate students)

- PYC 701- Neurobiology I: Lectures on Neurotransmitter Release, Synaptic Morphology, and Serotonin Neurochemistry.
- PYC 751- Neurochemical Pharmacology of Monoamine Neurons: Lectures on Protein Biochemistry and Physiological Regulation of Tyrosine Hydroxylase, Protein Biochemistry and Physiological Regulation of Tryptophan Hydroxylase, and Physiological Definition of Serotonin Neuronal Systems.

- PYC 756- Advanced Topics in Behavioral Pharmacology: Course Leader and Coordinator with lectures on operant control of behavior and the behavioral analysis of drug action, and behavioral and biochemical models of psychiatric diseases.

- PHC 750- Neuropharmacology I: Serotonin Neurochemistry and Neuropharmacology. Department of Pharmacology, Wayne State University School of Medicine.

- IBS 7050- Systems Biology-Neurobiology- Two credit hour course taught as part of the combined interdisciplinary biomedical curriculum in all School of Medicine PhD programs.

- PYC 7010- Molecular Neuropsychopharmacology- Lectures on pre-synaptic organization, essential elements of exocytosis and endocytosis, and vesicle structure; lectures on genetic polymorphisms and microarrays in neuropsychopharmacology.

- PYC 760 – Advanced topics course on emerging concepts in Parkinson's Disease and other neurodegenerative conditions with a focus on microglial activation and mediation as a cause of neuronal damage.

- PYC 7595 - The Gut Microbiome and Translational Neuroscience- starting Fall 2020 semester (Course director M. Angoa-Perez; co-director D.M. Kuhn)

Teaching at Wayne State (Residents/Fellows)

- Psychiatry Resident's Summer Seminar Program, 2016-present

**Mentorship**

| Name | Status | Dates | WSU/VA | Clinical or Basic Research | Current Position or Activity |
|---|---|---|---|---|---|
| William A. Wolf | Predoctoral | 1981-1985 (PhD) | WSU | Basic | Hines VAMC and Adjunct Professor, Department of Anatomy & Cell Biology, University of Illinois at Chicago, Chicago, IL |
| Patricia A. Johanson | Predoctoral (F31 funded) | 1990-1993 (PhD) | WSU | Basic | Senior Clinical Publications Lead, AstraZeneca Pharmaceuticals, Philadelphia, PA |
| Carroll M. D'Sa | Predoctoral | 1994-1996 (PhD) | WSU | Basic | Business Systems Analyst, Yale Center for Clinical Investigation, Yale University School of Medicine, New Haven, CT |
| Krishnamoorthy Sankaran, PhD | Postdoctoral | 1989-1991 | WSU | Basic | Head Chemist, City of Detroit, Dept. Water and Sewerage, Detroit, MI |
| Ulrike Berresheim, MD | Postdoctoral | 1990-1991 | WSU | Basic | Private medical practice, Anesthesiology and Pain Management, St. Ulrich a.P., Tirol, Austria |
| Ellen Zaija, MD | Postdoctoral | 1990-1991 | WSU | Basic | Private medical practice, Radiation Oncology, Milwaukee, WI |
| William A. Wolf, PhD | Postdoctoral | 1990-1992 | VA | Basic | Hines VAMC and Adjunct Professor, Department of Anatomy and Cell Biology, University of Illinois at Chicago, Chicago, IL |
| Barbara Gibbs, PhD | Postdoctoral | 1990-1991 | WSU | Basic | Senior Patent Attorney, Office of Technology Commercialization, Purdue University, West Lafayette, IN |
| Panos Z. Anastasiadis, PhD | Postdoctoral | 1994-1996 | WSU | Basic | Professor of Cancer Biology (Tenured), Mayo Clinic, Jacksonville, FL |
| Samuel U. Park | Predoctoral (F31 funded) | 1999-2007 | WSU | Basic | |
| Cheryl W. Aretha, PhD | Postdoctoral (F32 funded) | 1998-2000 | WSU | Basic | Professor, Biology Department, Macomb Community College, Macomb, MI |
| Mark Ritter, MD | Postdoctoral | 2000-2001 | WSU | Basic/Clinical | Resident, Psychiatry & Internal Medicine, WSU School of Medicine |
| Mahdieh Sadidi | Predoctoral | 1999-2004 (PhD) | WSU | Basic | Postdoctoral Fellow, Michigan State University, East Lansing, MI |
| Stacey (Sakowski) Jacoby | Predoctoral | 2000- | WSU | Basic | Deputy Managing Director, Alfred Taubman Medical |

| | | | | | |
|---|---|---|---|---|---|
| | | 2006 (PhD) | | | Research Institute, University of Michigan SOM, Ann Arbor, MI |
| David M. Thomas, PhD | Postdoctoral and NIH KO1 mentor | 2002-2005 | VA | Basic | Professor (Tenured), Department of Biological Sciences and Assistant Dean for Medical Education, Oakland University William Beaumont School of Medicine, Rochester Hills, MI |
| Pamela VandeVord, PhD | Mentor on VA Career Dev. Award | 2007-present | VA | Basic | Professor (Tenured), School of Biomedical Engineering and Sciences, Virginia Polytechnic Institute & State University, Blacksburg, VA |
| Alana Conti, PhD | Mentor on NIH KO1 | 2012-2014 | WSU/VA | Basic | Associate Professor (Tenured), Department of Neurosurgery, WSU School of Medicine |
| Michael J. Kane, PhD | Postdoctoral | 2011-2013 | WSU/VA | Basic | Adjunct Assistant Professor, Neuroscience Program, Temple University, Philadelphia, PA |
| Mariana Angoa-Perez, PhD | Postdoctoral | 2009-present | WSU/VA | Basic | Postdoctoral Research Associate, WSU School of Medicine |
| Nieves Herrera-Mundo, PhD | Postdoctoral | 2012-2014 | WSU/VA | Basic | Postdoctoral Fellow, Biological Sciences, National Autonomous University of Mexico, Mexico City MX |
| John H. Anneken, PhD | Postdoctoral | 2013-present | WSU/VA | Basic | Postdoctoral Research Associate, WSU School of Medicine |
| Denise I. Briggs, PhD | Pre- and Postdoctoral | 2012-2016 | WSU/VA | Basic | PhD, May 2016, Department of Neurosurgery, Stanford University School of Medicine |
| John A. Rotondo | Predoctoral | 2014-2015 | WSU/VA | Basic | Student in MD/PhD program, WSU School of Medicine |
| Denise I. Briggs, PhD | Pre- and Postdoctoral | 2012-2016 | WSU/VA | Basic | PhD, May 2016, Department of Neurosurgery, Stanford University School of Medicine |

**Theses and Dissertations directed**

- William A. Wolf, PhD dissertation, Studies on the Mechanisms which Regulate Serotonin Release, Department of Pharmacology, The George Washington University School of Medicine, June 1985.

- Patricia J. Johansen, PhD dissertation, Activation and Phosphorylation of Brain Tryptophan Hydroxylase by Protein Kinases, Cellular and Clinical Neurobiology Program, Wayne State University School of Medicine, August 1993.

- Carrol D'Sa, PhD dissertation, Regulation of Brain Tryptophan Hydroxylase, Cellular and Clinical Neurobiology Program, Wayne State University School of Medicine, July 1998.

- Mahdieh Sadidi, PhD dissertation, Molecular Footprints of Neurotoxicity: Posttranslational Modifications of Tyrosine Hydroxylase, Cellular and Clinical Neurobiology Program, Wayne State University School of Medicine, Dec. 2004.

- Stacey Sakowski, PhD dissertation, Biochemistry and Molecular Biology of Tryptophan Hydroxylase, Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, June, 2006.

- Denise I. Briggs, PhD Dissertation, Cognitive, Psychiatric and Neuropathological Outcomes of Repetitive Mild Traumatic Brain Injury, Translational Neuroscience Program, Wayne State University School of Medicine, PhD March 2016.

- John Rotondo, MS, Basic Medical Sciences, Wayne State University School of Medicine, 2014.

- Charles Fisher, MS, Basic Medical Sciences, Wayne State University School of Medicine, 2014.

- David Shaheen, MS, Basic Medical Sciences, Wayne State University School of Medicine, 2014.

- Julia Solarewicz, MS, Department of Physiology, Wayne State University School of Medicine, 2015

- Alhassan Dhia, MS, Basic Medical Sciences, Wayne State University School of Medicine, 2014.

- Helen Wu, MD/PhD Program, PhD Dissertation Committee member, Translational Neuroscience Program, Wayne State University School of Medicine, PhD May 2016.

- Muzamil Arshad, MD/PhD Program, PhD Dissertation Committee member, Translational Neuroscience Program, Wayne State University School of Medicine, PhD August 2016.

- Hamilton Trinh, M1 Honors Student thesis, Wayne State University School of Medicine, 2016.

- Krithika Muthkumaran, Department of Chemistry and Biochemistry, University of Windsor, External PhD Dissertation Examiner Sep 2016.

- Andrew Neff, Translational Neuroscience PhD Program, Dissertation Committee member, Wayne State University School of Medicine, PhD March 2018.

## GRANTS, CONTRACTS, AND OTHER FUNDING

### Active National/International Grants and Contracts

Role: Principal Investigator, Percent effort 20%, IK6RX002419
Title: Research Career Scientist Award
Source: Department of Veterans Affairs (VA) Rehab R&D
2006-2023
Total direct costs:

Role: Co-Principal Investigator, Percent effort 5%, PI: Jason Mateika
Title: 5HT modulation of arousal and chemoreflex responses in intact and SCI mice
Source: Department of Veterans Affairs (VA) Rehab R&D Merit Award
2018-2022
Total direct costs:

Role: Principal Investigator, Percent effort 20%, I01BX004340
Title: Delayed and Progressive Emergence of CTE- and Psychiatric-like Pathologies after Repetitive Mild TBI
Source: Department of Veterans Affairs (VA) Basic Laboratory R&D Merit Award
2019-2023
Total direct costs:

Role: Principal Investigator, Percent effort 20%, GW170034
Title: Gulf War Illness and Gut Microbiome Dysbiosis: Treatment with Probiotics and Fecal Transplantation
Source: Department of Defense, Congressionally Directed Medical Research Program
2019-2021
Total direct costs:

Role: Principal Investigator, Percent effort 20%, 1I01BX004757-01A1
Title: Gulf War Veterans' Illnesses: Symptom Chronicity via Interactions of Diet and Lifestyle Risk Factors with the Gut Microbiome
Source: Department of Veteran's Affairs (VA), Basic Laboratory R&D Merit Award
2020-2024
Total direct costs:

Role: Principal Investigator, Percent effort 10%, IS1 BX005515
Title: ShEEP Request for QIAGEN QiaCube Connect System
Source: Department of Veteran's Affairs (VA), Basic Laboratory R&D Merit Award
2020-2021
Total direct costs:

### Pending National/International Grants and Contracts

Role: Principal Investigator, Percent effort 20%,
Title: MDPV Abuse and the Gut Microbiome

Source: NIH, National Institute on Drug Abuse
2022-2024
Total direct costs:

**Previously funded Grants and Contracts**

Role: Principal Investigator, Percent effort 20%, I01RX000458
Title: Role of TPH2 and 5HT Neuronal Loss in Non-motor Symptoms of Parkinson's
Source: Department of Veterans Affairs (VA) Rehab R&D Merit Award
2016-2020
Total direct costs:

Role: Principal Investigator, Percent effort 5%, IS1BX004395
Title: ShEEP Request for an Illumina MiSeq System
Source: VA Office of Research & Development, Shared Equipment Award Program
2018-2019
Total direct costs:

Role: Principal Investigator, Percent effort 20%, R21DA034692
Title: βeta-ketoamphetamines: Window to the Neurotoxic Mechanisms of Methamphetamine
Source: NIH/NIDA Cutting Edge Basic Research Award
2015-2018
Total direct costs:

Role: Principal Investigator, Percent Effort: 100%, F32 HL0245
Title: Control Mechanisms for Serotonin Synthesis in Brain
Source: NIH/NHLBI
1976-1978
Total direct costs:

Role: Principal Investigator, Percent effort: 100%, NHLBI
Title: Intramural Research Program, Section on Biochemical Pharmacology, National Heart Lung & Blood Institute, National Institutes of Health
Source: NIH/NHLBI
1978-1986
Total direct costs: ~ (NIH Intramural funding)

Role: Principal Investigator, Percent effort: 10%
Title: Small Grant in Neurosciences Award
Source: Wayne State University School of Medicine
1986-1987
Total direct costs:
Role: Mentor, Percent effort: 5%
Title: Office of the Dean of the Medical School, Dean's Postdoctoral Recruiting Award (PI: Dr. W.A. Wolf)
Source: Wayne State University School of Medicine
1989-1990

Total direct costs:
Role: Principal Investigator, Percent Effort 20% R03 MH02365
Title: Tryptophan Hydroxylase: Purification and Production of Antibodies
Source: NIH/NIMH
1989-1990
Total direct costs:

Role: Principal Investigator, Percent effort 20%
Title: Differential Loss of Tyrosine Hydroxylase from the Striatum in Parkinson's Disease
Source: United Parkinson Foundation
1990-1991
Total direct costs:

Role: Principal Investigator, Percent effort 20%, R01 DA006219
Title: Cocaine and Serotonin Neurochemistry
Source: NIH/NIDA
1991-1995
Total direct costs:

Role: Principal Investigator, Percent effort 5%
Title: Small Instrumentation Grant Program
Source: Alcohol, Drug Abuse, and Mental Health Administration (administered through Wayne State University School of Medicine
1991-1992
Total direct costs:

Role: Mentor, Percent effort 5%
Title: Office of the Dean of the Medical School, Dean's Postdoctoral Recruiting Award (PI: Dr. B. Gibbs)
Source: Wayne State University School of Medicine
1992-1993
Total direct costs:

Role: Mentor, Percent effort 5%, F31 MH010230 National Research Service Award (Predoctoral)
Title: Tryptophan Hydroxylase: Regulation by Protein Kinases (PI: Patricia Johansen)
Source: NIH/NIMH
1992-1994
Total direct costs:

Role: Principal Investigator Percent effort 20%, R55 NS030833 (Shannon Award)
Title: Regulation of Brain Tryptophan Hydroxylase
Source: NIH/NINDS
1992-1995
Total direct costs:

Role: Principal Investigator, Percent effort 10%,
Title: Amphetamine Neurotoxins, 5-HT Neurons, and Nitric Oxide

Source: Wayne State University Office of Neuroscience Programs GETIN Grant
1993-1994
Total direct costs:

Role: Principal Investigator, Percent effort 5%
Title: Depression and Defects in Serotonin Neurochemistry
Source: Department of Psychiatry and Behavioral Neurosciences, Joe Young Sr. Research Grant
1994-1995
Total direct costs:

Role: Principal Investigator, Percent effort 5%
Title: Targeted Disruption of the Gene for Tryptophan Hydroxylase: Production of a Serotonin Deficient Knock Out Mouse as a Model for Psychiatric Disease
Source: Department of Psychiatry and Behavioral Neurosciences, Joe Young Sr. Research Grant
1994-1995
Total direct costs:

Role: Principal Investigator, Percent effort 10%
Title: Genetic Modification of Human Fibroblasts to Express Tyrosine Hydroxylase: Development of a Graft for Gene Therapy of Parkinson's Disease
Source: National Parkinson Foundation
1995-1997
Total direct costs:

Role: Principal Investigator, Percent effort 5%
Title: Neurotoxic Amphetamines, Radicals & 5HT Neurons
Source: NIH/NIEHS Center Grant (Center for Molecular and Cellular Toxicology with Human Applications Pilot Project; PI-Raymond F. Novak)
1996-1997
Total direct costs:

Role: Mentor, Percent effort 5%
Title: Office of the Dean of the Medical School, Dean's Postdoctoral Recruiting Award (PI: Dr. C. Aretha)
Source: Wayne State University School of Medicine
1999-2000
Total direct costs:

Role: Principal Investigator (Mentor), Percent effort 5%, R13 Conference Grant
Title: Neurotoxicity of Amphetamines and Related Stimulants
Source: NIH/NIDA and American Society for Pharmacology and Experimental Therapeutics
1999-2000
Total direct costs:

Role: Principal Investigator, Percent effort 10%
Title: Tyrosine hydroxylase as a cytotoxic protein in Parkinson’s disease
Source: Parkinson’s Disease Foundation

1999-2000
Total direct costs:

Role: Principal Investigator, Percent effort 5%
Title: Neuroprotective properties of pramipexole
Source: Pharmacia-Upjohn
1999-2000
Total direct costs:

Role: Principal Investigator, Percent effort 20% I01
Title: Neurotoxic Amphetamines, Proto-oncogenes, and Apoptosis
Source: Department of Veterans Affairs (VA) Basic Laboratory R&D Merit Award
1998-2002
Total direct costs:

Role: Principal Investigator, Percent effort 5%
Title: Paraquat, Dopamine-quinones & Parkinson's Disease
Source: NIH/NIEHS Center Grant (Center for Molecular and Cellular Toxicology with Human Applications Pilot Project; PI-Raymond F. Novak)
2000-2001
Total direct costs:

Role: Principal Investigator, Percent effort 10%
Title: Serotonin Knockout Model of Neurodevelopmental Disorders
Source: Children's Research Center of Michigan, Children's Hospital of Michigan, Wayne State University School of Medicine
2000-2002
Total direct costs:

Role: Mentor, Percent effort 5% F32 National Research Service Award (Postdoctoral)
Title: Molecular Markers of Methamphetamine Neurotoxicity (PI: Dr. C. Aretha)
Source: NIH/NIDA
2000-2002
Total direct costs:

Role: Principal Investigator, Percent effort 20%, R01 MH057743
Title: PKC Signaling and the Treatment of Bipolar Disorder
Source: NIH/NIMH
2000-2003
Total direct costs:

Role: Mentor, Percent effort 5% F31 DA006067 National Research Service Award (Predoctoral)
Title: The Role of Dopamine in Methamphetamine Toxicity (PI: Samuel Park)
Source: NIH/NIDA
2000-2003
Total direct costs:

Role: Principal Investigator, Percent effort 20%, R01 DA013753
Title: Microarray Analysis of Human Cocaine Addicts
Source: NIH/NIDA
2000-2004
Total direct costs:

Role: Principal Investigator, Percent effort 10%, T32 DA007310
Title: Neuroscience Training in Drug Abuse Training Grant
Source: NIH/NIDA
2000-2006
Total direct costs:

Role: Principal Investigator, Percent effort 20%, K05 DA014692
Title: Molecular Neurobiology of Drug Abuse Senior Scientist Career Development Award
Source: NIH/NIDA
2002-2007
Total direct costs:

Role: Principal Investigator, Percent effort 20%
Title: Microglia as Primary Mediators of Nerve Agent Neuropathy
Source: Department of the Army, Medical Chemical and Biological Defense Research Program
2006-2008
Total direct costs:

Role: Principal Investigator, Percent effort 20%, I01
Title: Brain Injury by Blast Overpressure: Role of Microglial Activation
Source: Department of Veterans Affairs (VA) Basic Laboratory R&D Merit Award
2007-2011
Total direct costs:

Role: Principal Investigator, Percent effort 20%, R01 DA017327
Title: Methamphetamine Neurotoxicity and Microglial Activation
Source: NIH/NIDA
2005-2012
Total direct costs:

Role: Principal Investigator, Percent effort 20%, R01 DA010756
Title: Neurotoxic Amphetamines, Radicals & 5HT Neurons
Source: NIH/NIDA
2002-2013
Total direct costs:

Role: Principal Investigator, Percent effort 20%, I01 RX000375
Title: TBI & Alcohol Abuse: Co-occurring Conditions that Enhance Brain Damage
Source: Department of Veterans Affairs (VA) Rehab R&D Merit Award
2012-2016
Total direct costs:

## PUBLICATIONS

### Peer-Reviewed Publications

1. **Kuhn, D.M.**, Greenberg, I., and Appel, J.B. Differential effects on lever choice and response rate produced by d-amphetamine. Bull. Psychonom. Sci. 3, 119-120, 1974.

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3. Greenberg, I., **Kuhn, D.M.**, and Appel, J.B. Behaviorally-induced sensitivity to the discriminable properties of LSD. Psychopharmacologia 43, 229-232, 1975.

4. Greenberg, I., **Kuhn, D.M**., and Appel, J.B. A comparison of the discriminative stimulus properties of $\Delta^9$-THC and psilocybin in rats. Pharmacol. Biochem. Behav. 3, 931-934, 1975.

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7. Shah, N.S., Hixon, B., Gulati, O.D., **Kuhn, D.M.**, and Mathur, P.P. Methaqualone: Tissue distribution in control and SKF 525-A-pretreated pregnant, non-pregnant female and male Mice. Toxicol. Appl. Pharmacol. 40, 497-509, 1977.

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9. Christoph, G.R., **Kuhn, D.M.**, and Jacobs, B.L. Electrophysiological evidence for a dopaminergic action of LSD: Depression of unit activity in the substantia nigra of the rat. Life Sci. 21, 1585-1596, 1977.

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11. **Kuhn, D.M**., Vogel, R., and Lovenberg, W. Calcium-dependent activation of tryptophan hydroxylase by ATP-magnesium. Biochem. Biophys. Res. Comm. 82, 756-766, 1978.

12. **Kuhn, D.M**. and Shah, N.S. Subcellular localization of methaqualone-$^{14}$C in mouse brain: Effects of hepatic microsomal enzyme inhibition. Toxicol. Appl. Pharmacol. 46, 109-116, 1978.

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14. Christoph, G.R., **Kuhn, D.M.**, and Jacobs, B.L. Dopamine agonist pretreatment alters LSD's electrophysiological action from dopamine agonist to antagonist. Life Sci. 23, 2099-2110, 1978.

15. Lovenberg, W. and **Kuhn, D.M.** Role of hydroxylase cofactor in serotonin synthesis. Psychopharm. Bull. 14, 44-46, 1978.

16. Shah, N.S. and **Kuhn, D.M.** Regional localization of methaqualone in rat brain. Res. Comm. Chem. Path. Pharmacol. 22, 593-596, 1978.

17. White, F.J., Appel, J.B., and **Kuhn, D.M.** Discriminative stimulus properties of quipazine: Direct serotonergic mediation. Neuropharmacology 18, 143-151, 1979.

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21. Sankaran, K., **Kuhn, D.M.**, and Lovenberg, W. Cyclic AMP specific, calcium independent phosphodiesterase from a malignant murine mast cell tumor. Biochem. Biophys. Res. Comm. 89, 793-799, 1979.

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33. **Kuhn, D.M.** and Lovenberg, W. Inactivation of tyrosine hydroxylase by reduced pterins. Biochem. Biophys. Res. Comm. 117, 894-900, 1983.

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58. Johansen, P.A., Jennings, I., Cotton, R.G.H., and **Kuhn, D.M.** Immobilization of tryptophan hydroxylase by immune adsorption: A method to study regulation of catalytic activity. Brain Res. Bull. 29, 949-953, 1992.

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47. Qiu, Q., Kariharan, T., **Kuhn, D.** and Mateika, J. Spinal cord injury leads to increases in apnea frequency and duration in spontaneously breathing mice with and without central nervous system 5HT , Experimental Biology Meeting, April, 2020.

48. Angoa-Perez, M, Zagorac, B., Mackle, J., Harris, A., Lunerti, V., Kuhn, B.N., Cannella, N., Solberg-Woods, L., Allen, A., Chung, D., Ubaldi, M., Kalivas, P., Ciccocioppo, R., Hardiman, G., **Kuhn, D.M.** Long access heroin self-administration significantly alters gut microbiome diversity, structure and composition. NIDA Genetics and Epigenetics Cross-Cutting Research Team (GECCRT) Meeting. March 2021.

49.

**Invited Lectures/Presentations (selected)**

1. Microglial activation and drug-induced neurotoxicity: Nexus between HIV- and methamphetamine-induced neuronal damage, Invited lecture, NIMH/NIDA Conference on HIV and Substance Abuse, Bethesda, MD, March 2006.

2. Methamphetamine-induced neurotoxicity: Cross-talk between microglia and dopamine nerve endings reveals novel mechanisms of drug-induced neuronal damage, Invited lecture, Department of Pharmacology, Boston University School of Medicine, Boston, MA, May 2006.

3. Microglial activation as a specific marker for neurotoxicity, Invited platform lecture, Experimental Biology, Washington, DC, August 2006.

4. Microglial-neuronal crosstalk: How the innate immune system of the CNS is tricked into damaging neurons, Invited lecture, Department of Anatomy and Cell Biology, Wayne State University School of Medicine, Detroit, MI, March 2007.

5. Modulation of dopamine neuronal function by peroxynitrite: Dopamine as a molecular switch between nitrosative and oxidative stress, Invited lecture, Sun Health Research Institute, Phoenix, AZ, April 2007.

6. Microglial-neuronal crosstalk: How the innate immune system of the CNS is tricked into damaging neurons, Invited lecture, Department of Neuroscience, Medical University of South Carolina, Charleston, SC, May 2007.

7. Regulation of serotonin function by TPH2: Protein kinases and the UPP interact to determine enzyme stability, Invited lecture, Department of Pharmacology and Toxicology, Michigan State University School of Medicine, East Lansing, MI, Nov. 2008.

8. Role of microglial activation in drug-induced neurodegeneration, Invited platform lecture, Experimental Biology, New Orleans, LA, April 2009.

9. The role of non-neuronal cells and dopamine in drug-induced neurotoxicity, Invited lecture, Institute of Biomedical Investigations, National Autonomous University of Mexico, Mexico City, Mexico, Aug. 2009.

10. The brain without serotonin: Targeted deletion of the TPH2 gene uncovers a complex physiological and behavioral phenotype, Invited lecture, Department of Pharmaceutical Sciences, Eugene Applebaum College of Pharmacy and Health Sciences, Detroit, MI, Nov. 2009.

11. Role of dopamine and non-neuronal cells in methamphetamine-induced neurotoxicity, Invited lecture, Oregon Health Sciences University and Portland VA, Portland, OR, Mar. 2010.

12. Serotonin and psychiatric illness: New views from mice lacking TPH2, Invited lecture, Institute of Environmental Health Sciences, Wayne State University, Detroit, MI, Nov. 2010.

13. TBI and alcohol comorbidity: Interactions that complicate long-term outcome, Invited Lecture, NIH/VA/DoD Interagency Conference on TBI, Washington, DC, Jun. 2011.

14. The emerging problem of repetitive mild traumatic brain injury: Basic research perspectives and challenges, Invited Lecture, Michigan Psychiatric Society, Lansing, MI, Nov. 2012.

15. Animal models of sports-related head injury: Bridging the gap between preclinical research and clinical reality, Invited lecture, Department of Neurosciences, University of Toledo College of Medicine and Life Sciences, Toledo, OH, Dec. 2013.

16. Repetitive mild TBI: Challenges for investigation, detection and treatment, Invited lecture, Department of Neurology, Henry Ford Health System, Detroit, MI, Feb. 2015.

17. Interactions between repetitive mild TBI and alcohol: Does intoxication alter neuropathological outcomes of head injury, Invited speaker, Research Society on Alcoholism, New Orleans, LA, Jun. 2016.

18. Life Without Brain Serotonin: A New Look at an Old Neurotransmitter, Invited seminar, Department of Chemistry and Biochemistry, University of Windsor, Windsor, Ontario, Canada, Nov. 2016.

19. Synthetic Psychoactive ("bath salts") Drugs and Neurotoxic Amphetamines: Chemical Relatives with Very Different Modes of Action on the Brain, Invited Seminar, Department of Pharmacology, School of Pharmacy, University of Toledo, Toledo, OH, Feb. 2017.

20. Synthetic Psychoactive ("bath salts") Drugs and Neurotoxic Amphetamines: Chemical Relatives with Very Different Modes of Action on the Brain, Invited symposium speaker, International Behavioral Neuroscience Society Annual Meeting, Hiroshima, Japan, June 2017.

21. Gulf War Veterans' Illness: Symptom Chronicity via Interactions of Diet and Lifestyle Risk Factors with the Gut Microbiome. CDMRP-VA State of the Science Conference (Virtual) on Gulf War Illness, August 18-19, 2020.