Dear Celebration Committee,

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Meeting (ENDO 2016) with me April 1-3 in Boston, MA. Our abstract, entitled ò • † ‘ ’ Ž ƒ • • ‹ …
Reticulum Stress Alters the Transcriptional Profile of GnRH-Producing GT1-7 y ‡ Ž Ž • á ó ™ ƒ • ƒ … …
for poster presentation in January. Noah Levi, Jared Santana, Graham Redweik, and Zack Campbell each made valuable contributions to the design and execution of the experiments we will present, as well as to data analysis and figure generation. While the hands-on laboratory work these students have done is a critical component of their science education, learning how scientists communicate their work is also essential to their understanding of the process of science. I also strongly believe that our students deserve exposure to research at multiple levels and that we as educators should encourage their engagement in the world outside of Wabash. Attending a national research meeting like ENDO 2016, my students will gain valuable experience with scientific communication while at the same time be exposed to cutting-edge research from top-tier institutions from all over the world.

ENDO 2016 is the 100th annual meeting of The Endocrine Society, an internationally recognized professional society that promotes basic, translational, and clinical research in endocrinology. I have attended this meeting multiple times as a graduate student and post-doctoral fellow, and have always had extremely positive experiences. This meeting is somewhat unique because it bridges the gap between basic research and clinical work. Since each of my students are considering careers either in biomedical research or medicine, I believe they too will leave feeling energized about how research like ours can make a larger impact. This meeting also has multiple opportunities for participants at all levels (including undergraduates) to network with other scientists; but, 244i1H Ip] TJ ET BT 1 0 0 1 eN80.1brm02hut1nt6i6scien- ing represents

an opportunity for these students to interact with leaders in our sub-specialty of reproductive neuroendocrinology and get feedback on the scientific merit of our work.

The accepted abstract combines work from all four students to create a • – ” ‘ • ‰ ‡ ” • … ‹ ‡ • – ‹ ^ ‹ … ò
and as their mentor I will work with the students as we design our poster to ensure they each understand how all of the parts come together. Furthermore, I will be on-hand during our poster session to help them field difficult questions, but based on their recent presentations at the Research Celebration, I am confident that these four students will represent Wabash wonderfully. Finally, this will be the first meeting I attend and present my work at since establishing my own research program. Thus, I am excited for the opportunity to provide exposure for Wabash College at a new national venue, and hope that this meeting will forge new academic collaborations for me and the College.

In closing, I am grateful for the committee ï • … ‘ • • ‹ † ‡ ” ƒ – ‹ • ‘ ^ • › • – — † ‡ • – • ï ’ ” ‘ ’ ‘ • ƒ Ž -
ENDO 2016. Below you will find our accepted abstract, statements from all four students, and a

budget of estimated travel costs. Please feel free to contact me should you need any additional information .

Sincerely,

Heidi E. Walsh

Endoplasmic Reticulum Stress Alters the Transcriptional Profile of GnRH-Producing GT1-7 Cells.

Noah J. Levi, Jared R. Santana, Graham Redweik, Zachery R. Campbell, Heidi E. Walsh

Obesity islinked to infertility, but the impact of obesity-induced metabolic changes on the reproductive axis is unclear. In mammals, reproductive function is regulated by hypothalamic gonadotropin-releasing hormone (GnRH), which controls pituitary gonadotropin release and subsequent gonadal function. Dysfunction of GnRH-producing neurons leads to infertility in both sexes. The excess of circulating nutrients generated from overnutrition interferes with the function of hypothalamic neurons that control food intake by disrupting normal protein folding in the endoplasmic reticulum (ER). When protein folding load exceeds cellular folding capacity, the cell initiates an ER stress response (the unfolded protein response, or UPR) through a defined set of signaling pathways and transcription factors. In obesity, ER stress can also promote inflammatory responses in hypothalamic neurons, creating a vicious cycle of cellular dysfunction. Using the immortalized mouse hypothalamic cell line GT1-7 as a model, we measured three important transcriptional targets (*Fos, Il6,* and *Gnrh1*) to determine how fertility may be impacted by ER stress.The transcription factor c-fos, encoded by the *Fos* gene,is implicated in protein kinase C (PKC)-induced downregulation of *Gnrh1* as well as upregulation of the pro-inflammatory cytokine *Il6* by inflammatory signals. In GT1-7 cells, ER stress (induced by tunicamycin or thapsigargin) increased *Fos* expression in a PKC-dependent manner, as pretreatment with the broad-spectrum inhibitor Gö6983 blocked *Fos* induction. ER stress induced a canonical UPR in GT1-7 cells, as measured by increased *Ddit3* and *CEBPB* mRNA levels, as well as an inflammatory response, as evidenced by increased *Il6* mRNA. Stress.

My involvement with the project began this previous summer. Dr. Walsh took me into her lab for a summer internship; upon which, my colleagues and I were given individual projects in efforts to piece the interaction of ER stress and GnRH levels in GnRH neurons together. Given my previous exposure to inflammatory pathways from my Endocrinology course, Dr. Walsh and I thought it would be fitting for me to take on this portion of the project. In other words, my role became investigating the inflammatory pathways ER stress was inducing to increase inflammatory signals. During the summer, I ran multiple experiments analyzing signaling pathway activity and gene expression. This portion of the project was quite interesting as an unexpected conclusion was

graduate programs afterWabash. Additionally, I feel as though my contribution to the research is sound, and that this Endocrine Society meeting would provide me with a unique ability to see what other areas and questions endocrinology research is investigating. I would be extremely grateful to be given the support and opportunity to make this trip to Boston with my Professor and colleagues.

[illegible] assisted in adding another piece to the puzzle in our attempt to understand how obesity is related to infertility. We knew prior to the research that obesity increases levels of reactive oxygen species in gonadotropin-releasing hormone (GnRH) neurons, which induces endoplasmic reticulum (ER) stress in cells. This ER stress, or unfolded protein response, causes a cellular cascade that inevitably prevents normal GnRH neurons from functioning properly, leading to infertility. However, the exact steps in this process are unclear, and my research helped provide additional insight into the mechanism of this pathway. I found that protein kinase C (PKC), a regulatory protein found in several tissue types, mediated ER stress by increasing levels of IL-6 (pro-inflammatory cytokine) and c-fos (transcription factor) gene expression in GnRH neurons. I am continuing work on this project in the lab this semester, and thus attending ENDO 2016 would be extremely valuable because I will get feedback and new ideas about our work.

The opportunity to present our research in Boston would be extremely beneficial to both my academic and aspirational development. Seeing how other people have gone about researching neuroendocrine pathways (even different from ours) would provide valuable insight and could potentially create new ways for us to more efficiently conduct our research. Additionally, observing findings from other researchers would benefit my development as a scientist, enabling me to become more knowledgeable in how the human body works. This is very important to me because I want to conduct research that could be utilized in a clinical setting, and having a greater base of knowledge would only enhance my ability to relate my research to ways to help people facing disease. Being in a setting with many graduate students as well as experts in their respective fields would provide a great opportunity for me to make connections with people I will not have had the opportunity to do so without this experience. This is especially important for me as I have currently applied to Ph.D. programs in Biology. Specifically, some of the talks I am [illegible] are researching, and these would give me a better understanding of how the brain mediates the endocrine system. Hopefully, I will have the pleasure of going to such an acclaimed research event, further reaffirming my interest to be involved in biological research as a career.

Obesity is a health concern that often leads to increased risk of other ailments such as infertility. Gonadotropin- Releasing Hormone (GnRH) is a protein that is expressed in specific neurons in the hypothalamus and is a key regulator of fertility. GnRH expression is stimulated by the neuropeptide Kisspeptin. Obesity causes stress on the Endoplasmic Reticulum (ER). When stress is placed on the system, the Unfolded Protein Response (UPR) is activated to counteract the stress.

We focused on understanding how GnRH neurons and their response to Kisspeptin is impacted by ER stress. I treated cultured GT-1 mouse hypothalamus cells with Kisspeptin and the ER stress inducer, Tunicamycin, to determine the respective interactions between these signals, the UPR, and Gnrh1 gene expression. Quantitative RT-PCR studies confirmed that tunicamycin adversely affected the production of GnRH, while Kisspeptin increased GnRH production. There was also a trend seen between tunicamycin and the up regulation of expression of the Slc12a2 gene, which encodes a chloride transporter essential for GnRH neuron function. Future work will determine if effects on Slc12a2 are significant and whether ER stress can block the induction of Gnrh1 by Kisspeptin.

The ENDO2016 conference in Boston provides a unique experience for those of us who worked in Dr. Walsh's lab this summ‡ ” ƒ • ™ ‡ Ž Ž ƒ • ƒ ˜ ƒ Ž — ƒ „ Ž ‡ ‰ ƒ – ‡ ™ ƒ › – Biology ” ‡ • ‡ • – department. Over the three day period we would have substantial exposure to other researchers, physicians, and students, granting us the ability to expand our network of contacts. The opportunities for networking with a wide variety of people in top of the line science fields would be invaluable for our career paths. As students of science, effective communication with others in the scientific community is extremely important. The ENDO2016 conference gives us a prime setting to refine the craft of presenting in a professional atmosphere, helping to give us a competitive edge in our post-graduation plans. We would gain insightful feedback about our research by others in the field while also showing the top notch research that we conduct at Wabash. We would be able to attend other events such as debates, interview sessions, and step sessions on various topics related to our own line of work which would widen our own scope of knowledge on endocrinology.

The opportunity to present at ENDO 2016 would be an invaluable experience for me and the others who worked ‹ • ” ä ƒ Ž • Š ï • Ž ƒ „ ä • • ‘ • ‡ ‘ • ‡ ™ Š ‘ ™ ƒ • – • – ‘ ‰ ‘ ‹ • – ‘ • ‡ † many of the concepts that will be presented upon have implications in the medical field and are critical to my career aspirations. The chance to network, develop professional skills, and spend a weekend learning about Endocrinology from premier sources would be a cornerstone in my research experience and career at Wabash.

ENDO 2016