BIOGRAPHICAL SKETCH

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NAME: HOLLAND, RUBY

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POSITION TITLE: MD/PhD Student

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE (if applicable)	END DATE MM/YYYY	FIELD OF STUDY
University of Pennsylvania, Philadelphia, PA	BA	05/2016	Chemistry
University of Pittsburgh School of Medicine -- Carnegie Mellon University, Pittsburgh, PA	PHD	05/2023	Neurobiology
University of Pittsburgh School of Medicine, Pittsburgh, PA	MD	05/2025	Medicine

A. Personal Statement

I am currently an MD/PhD candidate at the University of Pittsburgh School of Medicine and Carnegie Mellon University MSTP. My career goal is to become a physician-scientist in academic anesthesiology, applying my insights from long-term pain management in the clinic to my research interests in neurophysiological mechanisms of pain signaling. Throughout my training, I have been motivated by the impact of basic science research on patient quality of life. This began as early as my freshman year of college, when I began working as a research assistant in a nutritional neuroscience lab with Dr. Bart De Jonghe. Through my work studying the neural pathways implicated in chemotherapy-induced anorexia and malaise, I learned a variety of technical skills such as working with laboratory rats and mice, immunohistochemistry, PCR, and other tissue processing techniques. Dr. De Jonghe encouraged me to take on independent projects, contribute to manuscripts from early on in my training, and communicate my work with poster presentations. While this work was solely preclinical, the potential applications of this research on medicine became apparent to me while fundraising for the American Cancer Society and volunteering in a cancer unit at the Hospital of the University of Pennsylvania, I met patients whose severe nausea dominated their lives inside and outside of the hospital. After graduating from the University of Pennsylvania, I continued to work with Dr. De Jonghe, taking on a full-time technician position which entailed more responsibility and freedom to design experiments. These experiences solidified my passion for symptom management and quality of life, and how my research could benefit these endeavors through informing the development of targeted antiemetics.

I became interested in pain research during a collaboration with Drs. Amber Alhadeff and Nicholas Betley investigating the integration of competing hunger and pain signals in the lateral parabrachial nucleus. In this set of projects, I conducted pain experiments utilizing novel behavioral paradigms, chemogenetics, and tissue processing techniques. This research experience inspired me to apply to the University of Pittsburgh MSTP with the vision of conducting my graduate work at the Center for Pain Research. This core of faculty consists of many exceptional MSTP mentors who are experts in pain research, including Dr. Sarah Ross and Dr. Michael Gold, both of whom I have worked with on central and peripheral mechanisms of pain. During my first two years of medical school I took active steps to pursue my interest in anesthesiology by taking on a leadership role in the Anesthesiology Interest Group, enrolling in a peripheral nerve block elective course, and shadowing multiple anesthesiologists. In my OB/GYN clerkship course, I also actively sought out opportunities to care for patients with pelvic pain conditions. Through these experiences I have become acquainted with an expansive community of anesthesiologists and neuroscientists who share my passion for pain management and improving quality of life. I believe that pursuing an M.D./Ph.D at the University of Pittsburgh, alongside award-winning investigators and passionate clinicians will prepare me to take significant steps towards my goal of becoming an independent investigator in academic anesthesiology.

B. Positions and Honors

ACTIVITY/ OCCUPATION	START DATE	ENDING DATE	FIELD	INSTITUTION/ COMPANY	SUPERVISOR/ EMPLOYER
Undergraduate Research Assistant	09/2012	05/2016	Nutritional Neuroscience	University of Pennsylvania	Dr. Bart De Jonghe, PhD
Research Technician/Lab Manager	06/2016	05/2017	Nutritional Neuroscience	University of Pennsylvania	Dr. Bart De Jonghe, PhD
Teaching Assistant	07/2015	05/2016	Chemistry	University of Pennsylvania School of Nursing	Dr. Antonio Davila, PhD
PhD Student	07/2019	06/2023 (anticipated)	Neurobiology	University of Pittsburgh	Dr. Sarah Ross, PhD

Academic and Professional Honors

2012-2013 Dean's List, University of Pennsylvania
2015 Lt. Col. M. Richman Scholarship, University of Pennsylvania
2016 Graduated *cum laude*, University of Pennsylvania
2019 Best Workshop Award, University of Pittsburgh MSTP

Academic Activities

2014-2015 Pre-Medical Volunteer, Hospital of the University of Pennsylvania
2014-2015 Webmaster, Penn Colleges Against Cancer, American Cancer Society
2015-2016 Entertainment Committee Chair, Penn Colleges Against Cancer, American Cancer Society
2015-2016 President, Stitch for Kids, University of Pennsylvania
2018-2018 MSTP Second Look Committee, University of Pittsburgh
2018-2018 MSTP Welcoming Committee, University of Pittsburgh
2018-2018 MSTP Hosting Committee, University of Pittsburgh
2018-Present Coordinator, Anesthesiology Interest Group, University of Pittsburgh
2018-Present Coordinator, Knitt Med, University of Pittsburgh
2019-Present Wellness Committee Member, University of Pittsburgh

Research Support

2013 Penn Undergraduate Research Mentorship Grant, University of Pennsylvania
2013 Biology and Control of Nausea and Vomiting 2013 Conference Travel Award, Cyclic Vomiting Syndrome Association
2014 University Scholars Summer Stipend, University of Pennsylvania
2015 University Scholars Summer Stipend, University of Pennsylvania
2015 University Scholars Travel Grant, University of Pennsylvania
2017-2019 T32 Grant, Medical Scientist Training Program, University of Pittsburgh
2019-Present R01 Diversity Supplement, University of Pittsburgh

C. Contribution to Science

1. **Characterization of glutamate receptor signaling implicated in chemotherapy-induced anorexia and malaise**

Cisplatin chemotherapy is used commonly to treat a variety of cancers despite severe side effects such as nausea, vomiting, and anorexia that compromise quality of life and limit treatment adherence. The neural mechanisms mediating these side effects remain elusive. Because glutamate signaling in the hindbrain is implicated in inhibitory feeding pathways, the De Jonghe lab investigated glutamatergic circuits implicated in chemotherapy-induced malaise and anorexia in rodent models. I was an undergraduate student when I contributed to the discovery that cisplatin activates neurons within the nucleus tractus solitarius (NTS), lateral parabrachial nucleus (lPBN), central nucleus of the amygdala (CeA), and bed nucleus of the stria terminalis (BNST) in rats. Using PCR, we determined cisplatin induces increased AMPA and NMDA receptor subunit expression in all of these regions. We then demonstrated through local injections of glutamate receptor

antagonists and food intake measurements that CeA glutamate receptor signaling mediates cisplatin-induced anorexia and body weight loss. Together, these findings help to characterize the neural mechanisms mediating cisplatin-induced anorexia, advancing opportunities to develop better-tolerated chemotherapies and adjuvant therapies to prevent anorexia and concurrent nutritional deficiencies during cancer treatment. My contributions to this work included experimental design, behavioral experiments on rats and mice, stereotaxic surgeries, as well as tissue harvesting and processing. I was also extensively involved in data analysis and preparation of manuscripts.

a. **Holland RA**, Leonard JJ, Kensey NA, Hannikainen PA, De Jonghe BC (2014). Cisplatin induces neuronal activation and increases central AMPA and NMDA receptor subunit gene expression in mice. Physiol Behav. 2014 Sep;136:79-85. PubMed PMID: 24582677.

b. Alhadeff AL, **Holland RA**, Nelson A, Grill HJ, De Jonghe BC (2015). Glutamate Receptors in the Central Nucleus of the Amygdala Mediate Cisplatin-Induced Malaise and Energy Balance Dysregulation through Direct Hindbrain Projections. J Neurosci. 2015 Aug 5;35(31):11094-104. PubMed PMID: 26245970; PubMed Central PMCID: PMC4524978.

c. **Holland RA**, De Jonghe BC (2013). Cisplatin induces a dose-dependent increase in Fos expression in the mouse brain. Center for Undergraduate Research and Fellowships Open House, University of Pennsylvania. Poster Presentation. Philadelphia, PA.

d. **Holland RA**, Leonard JJ, Kensey K, Hannikainen, P, De Jonghe BC (2013). Anorectic doses of cisplatin induce neural activation and alterations in AMPA and NMDA receptor subunit gene expression in mice. Biology and Control of Nausea and Vomiting Conference. Poster Presentation. Pittsburgh, PA.

2. **Investigation of a hindbrain-forebrain circuit involved in cisplatin-induced anorexia and weight loss.**

Previous work from the De Jonghe lab identified three major brain regions activated by injection of cisplatin chemotherapy: the NTS, lPBN, CeA, and BNST. However, the connectivity between these brain regions and the role of these projections in anorexia and weight loss behaviors, however, was unknown. Therefore, we sought to determine the neuroanatomical connections between brain regions involved in chemotherapy-induced anorexia and weight loss and elucidate the role of these projections in rodent behaviors. Using the retrograde tracer Fluoro-Gold and the anterograde tracer Fluoro-Ruby, we found cisplatin-induced neuronal activation in projections from the NTS to the PBN, as well as projections from the PBN to the CeA. Utilizing chemogenetic approaches, we then discovered that chemogenetic inhibition of lPBN to CeA projections attenuated cisplatin-induced anorexia and body weight loss in rats. Together, these experiments build on previous work by the De Jonghe lab and identify a hindbrain to forebrain circuit which is required for cisplatin-induced anorexia and weight loss in rats. My specific contributions to this project include assisting with stereotaxic injections, conducting behavioral experiments, tissue harvesting, and immunohistochemistry.

a. Alhadeff AL, **Holland RA**, Zheng H, Rinaman L, Grill HJ, De Jonghe BC (2017). Excitatory Hindbrain-Forebrain Communication Is Required for Cisplatin-Induced Anorexia and Weight Loss. J Neurosci. 2017 Jan 11;37(2):362-370. PubMed PMID: 28077715; PubMed Central PMCID: PMC5242394.

b. De Jonghe BC, **Holland RA**, Olivos DR, Rupprecht LE, Kanoski SE, Hayes MR (2016). Hindbrain GLP-1 receptor mediation of cisplatin-induced anorexia and nausea. Physiol Behav. 2016 Jan 1;153:109-14. PubMed PMID: 26522737; PubMed Central PMCID: PMC4862654.

c. **Holland RA** (2015). Neural Circuits of Nausea: Amygdala glutamate signaling in chemotherapy-induced nausea. University Scholars Talk. Philadelphia, PA.

d. **Holland RA**, Zimmer DJ, Hayes MR, De Jonghe BC (2015). Hindbrain neuroinflammation mediates cisplatin-induced pica and anorexia in the rat. Biology and Control of Nausea and Vomiting Conference. Poster Presentation. Pittsburgh, PA.

3. **Discovery of a neural circuit for the suppression of pain during hunger.**

Hunger and pain are two competing signals that individuals must resolve to ensure survival. However, the neural processes that prioritize conflicting survival needs are poorly understood. While working as a research technician, I contributed to a collaborative project investigating the effect of hunger on pain responses in rodents. My primary contribution to this project involved conducting rodent experiments and injections, tissue collection and processing, and optimizing an immunohistochemistry protocol for co-staining AgRP and NPY in mouse brain sections. We discovered that hunger attenuates behavioral responses and affective properties of inflammatory

pain without altering acute nociceptive responses. This effect is centrally controlled, as activity in hunger-sensitive agouti-related protein (AgRP)-expressing neurons abrogates inflammatory pain. Systematic analysis of AgRP projection subpopulations revealed that the neural processing of hunger and inflammatory pain converge in the hindbrain parabrachial nucleus (PBN). Strikingly, activity in AgRP → PBN neurons blocked the behavioral response to inflammatory pain as effectively as hunger or analgesics. The anti-nociceptive effect of hunger is mediated by neuropeptide Y (NPY) signaling in the PBN. By investigating the intersection between hunger and pain, we have identified a neural circuit that mediates competing survival needs and uncovered NPY Y1 receptor signaling in the PBN as a target for pain suppression. My primary contribution to this project involved conducting rodent injections, tissue collection and processing, and optimizing an immunohistochemistry protocol for co-staining AgRP and NPY in mouse brain sections.

a. Alhadeff AL, Su Z, Hernandez E, Klima ML, Phillips SZ, **Holland RA**, Guo C, Hantman AW, De Jonghe BC, Betley JN. A Neural Circuit for the Suppression of Pain by a Competing Need State. Cell. 2018 Mar 22;173(1):140-152.e15. PubMed PMID: 29570993; PubMed Central PMCID: PMC5877408.

4. **Investigation of a rat model of chemotherapy-induced peripheral neuropathy and cognitive impairments**

Paclitaxel is a chemotherapy commonly paired with carboplatin in the treatment of a variety of solid tumor cancers. Under this regimen, patients report an array of debilitating peripheral neurological symptoms including chemotherapeutic-induced peripheral neuropathy (CIPN) often experienced as a numbness and tingling in the hands and feet which develops into debilitating pain. In my first lab rotation in the Pittsburgh Center for Pain Research, I worked in the Gold Lab to study the mechanisms underlying paclitaxel-induced peripheral neuropathy and cognitive impairments. We hypothesized that the cognitive impairments caused by chemotherapy were due, in part, to changes in the gut microbiome, based on prior studies linking microbiome dysregulation to cognitive function. I contributed to this project by administering a paclitaxel/carboplatin cocktail to male and female rats and testing thermal and mechanical pain responses periodically over two months. I then measured memory through open field tests and anxiety through open field tests. Rats were then euthanized, and fecal samples and peripheral nerves were collected. Sex was found to be an important biological variable in this study, as my experiments showed female mice experienced significant increases in mechanical and thermal hyperalgesia as well as anxiety while males did not. This finding was consistent with human data indicating women report more severe symptoms of paclitaxel chemotherapy, indicating that rats were a viable model for paclitaxel-induced cognitive impairments.

a. **Holland RA,** Farias JP, Loeza-Alcocer E, Hartung J, Gold MS (2017). The Gut Microbiome: A Therapeutic Target for Paclitaxel-Induced Peripheral Neuropathy and Cognitive Impairments? Medical Scientist Training Program Annual Retreat. Poster Presentation. University of Pittsburgh MSTP. Pittsburgh, PA.

5. **Characterization of primary afferent phenotypes implicated in sexual pleasure and pelvic pain signaling.**

In my second lab rotation in the Pittsburgh Center for Pain Research, I worked in the Ross Lab to investigate a new frontier in somatosensory signaling: sexual pleasure. I used immunohistochemistry, neuroanatomical tracing, and a viral/genetic approach to visualize and characterize genital corpuscles, a specialized form of afferent which had not yet been identified in the mouse. I visualized two broad categories of primary afferents: a significant population of unique, clustered terminals within the glans of the mouse penis and clitoris, resembling genital corpuscular receptors described in prior literature, as well as free nerve endings extending to the epidermis. The genital corpuscular receptors are VGlut2 positive, identifying a genetic target by which these cells can be optogenetically or chemogenetically targeted to elucidate their role in sexual reward pathways.

a. **Holland RA**, Ross SE (2018). Neuroanatomical Tracing and Immunohistochemical Phenotype of the Genital Corpuscular Receptors of the Mouse Penis. Medical Scientist Training Program Annual Retreat. Poster Presentation. University of Pittsburgh MSTP. Pittsburgh, PA.