Sponsor and Co-Sponsor Statements

A. Research Support Available

ONGOING RESEARCH SUPPORT

Sarah Ross

R01 AR063772 06 — Ross (PI) 04/01/18 – 01/31/23
Investigating the neural circuits of itch

R01 NS096705 02 — Koerber (PD/PI) 9/01/16 – 8/31/21
Molecular genetic dissection of the spinal microcircuits of wind-up (Role: co-PI)

R01 EY029323 01 — Demb (PD/PI) 9/16/16 – 9/15/21
Functional circuitry of the long-range connections in the retina (Role: co-PI)

ADRC — Ross (co-PI) 4/01/16 – 3/01/20
The role of neurovascular dysfunction in the development of Alzheimer's Disease

H. Richard Koerber

R01 NS096705 02 — Koerber (PD/PI) 9/01/16 – 8/31/21
Molecular genetic dissection of the spinal microcircuits of wind-up (Role: co-PI)

R01 AR069951-02 — Koerber (MPI) 4/01/16 – 3/31/21
Characterization of Epithelial-Neural Communication

B. Sponsor's/Co-Sponsor's Previous Fellows/Trainees

Dr. Sarah Ross:

I take mentorship very seriously and work very hard to ensure the success of all my trainees. Two of the four students that have trained in my lab have already completed their graduate studies and the other two are on a track to complete within 4 – 4.5 years. **Lindsey Snyder** defended in July, 2017, with 8 peer-reviewed publications, including a first-author report in *Neuron*. She is currently pursuing postdoctoral training with Dietrich Stephan at the University of Pittsburgh. **Michael Chiang**, an MD-PhD student, defended July, 2019 with 4 peer-reviewed publications and a fifth, the major work from his thesis (available at *BioRxiv*), to be resubmitted next month first-author publication, having received strong reviews from *Neuron*. He is now returning to medical school. **Catherine Ruff**, who is beginning her third year, has made a major discovery about the regulation of cortical blood flow by long range inhibitory interneurons that express NK1R. She has already received two poster prizes for her work at internatisnal conferences. **Eileen Nguyen**, who is beginning her second year, received an NRSA to investigate the neural basis of morphine-induced itch. Since this project required a lot of breeding (and waiting for mice) she developed a back-up project studying the RVM neurons that express the kappa opioid receptor (putative OFF-cells). Now she has extremely exciting data from both projects and will likely graduate with two major first-author papers. All four of my current and previous graduate students received NRSAs and then went on to be highly successful with their scientific projects.

My first post-doc, **Junichi Hachisuka**, developed a novel preparation to study spinal circuitry (*Elife*) and used this preparation to investigate wind-up (*Pain*) and the neural circuits of cold (under review at *Pain,* available at *BioRxiv*). Based on his postdoctoral success with co-mentorship from me and Rick Koerber, he was hired as tenure-track faculty at the University of Glasgow (senior lecturer), which he started July, 2019. My second post-doc, **Marissa Kuzirian** received an NRSA, published several papers, and is now an Executive Associate at Pittsburgh Life Sciences Greenhouse.

Dr. H. Richard Koerber:

I have been the primary supervisor for 14 postdocs and 3 Ph.D. students, including an MD/PhD student.

9 of my former postdocs currently hold academic appointments (Karoly Mirnics, University of Nebraska; Jeffrey Woodbury, University of Utah, Michael Jankowski; Cincinnati Children's Hospital; Jeffrey Lawson, Fairmont

State; Sabrina McIlwrath, University of Kentucky; Kristofer K Rau, University of Louisville; Kyle Baumbauer, University of Connecticut; Bin Feng**,** University of Connecticut; Junichi Hachisuka, University of Glasgow).

4 of my former postdocs currently hold industry positions (Maggie Wright, Ultragenyx; Colleen Cassidy LifeX; Peter Adelman, Afinini; Amy Ritter Bristol-Myers Squibb).

One of my former Ph.D. students (Deepak Soneji) is currently Neurology Resident at UPMC.

C. Training Plan, Environment, Research Facilities

C.I *Training Plan*

Ruby Holland's goal is to become physician-scientist, where she will combine an independent research program to study pain and clinical work aimed at helping patients who suffer from pain. For her thesis work, she plans to investigate the descending neurons in the brainstem that enable top-down control of pain, and the pathological changes that occur within these circuits that may lead to chronic pain. The training described here is designed to allow Ruby to obtain all the technical and professional skills necessary to help enable this goal.

Rick Koerber and I are ideal co-mentors for Ruby because we are scientific partners, and we more or less run a joint lab with a joint lab meetings and daily interactions. In particular, Rick and I have an extensive and ongoing collaboration that includes four published papers, two additional manuscripts under review (uploaded to BioRxiv), an R21 (now completed) and an R01. As a testament of our success as a training team, our co-mentorship of Junichi Hachisuka resulted in his recent attainment of a faculty position as a senior lecturer at the University of Glasgow with the spinal cord group.

I am an expert in both pain and molecular genetic approaches to study neural circuit function, including the use of Cre alleles and viruses to target and manipulate specific neuronal populations. In my last eight years at the University of Pittsburgh, I have already developed a track record as an outstanding mentor to students and postdocs alike. Attesting to this, I was recently short-listed for William E. Brown Outstanding MSTP Mentor Award. Rick Koerber has been studying neural circuits of pain for the last 40 years, and has extensive experience with anatomy and electrophysiological recordings. Thus, for Ruby's training, I will be primarily responsible to ensure the success of Aims 1 (viral labeling, FISH, immunohistochemistry) and 3 (acute and persistent pain models), whereas Rick will be primarily responsible to ensure the success of Aim 2 (electrophysiology in slice). Together, our years of experience and expertise investigating somatosensory circuits will provide excellent mentorship for Ruby as she studies the neural circuits of descending modulation.

The training plan that Ruby and I have discussed has three key components: 1) mentored research training; 2) broad exposure to neuroscience and related fields through seminars, journals clubs, and attendance at national and international meetings; and 3) professional development activities.

1) Mentored research training

Ruby's training will consist largely of mentored research experience. Rick Koerber and I are very excited about her project, which we believe will provide important new insight into the neural circuits through which pain is modulated by descending circuitry in the RVM. In particular, Ruby will target the subset of MOR-expressing RVM neurons that project to the spinal cord, which are putative ON-cells, using a newly developed Oprm1-cre allele that was developed in the Palmiter lab **(Letter of Support)** and targeted viral delivery. The targeting of specific descending projections in this way will allow her to visualize and characterize a defined population of neurons (putative ON-cells), ascertain their targets in the spinal cord through optogenetics and electrophysiology, and then manipulate their activity in vivo (both activating and inhibiting) to provide cause-and-effect evidence for a role of these neurons in the modulation of pain. This combination of approaches will provide Ruby with many opportunities to expand her technical and methodological training, including viral labeling, stereotaxic surgeries, FISH, immunohistochemistry, cell quantification, electrophysiology, optogenetics in vitro, and behavioral experiments in vivo.

Ruby's training will be gained through the course of performing experiments, overseen primarily by myself and Rick Koerber. Rick and I are both highly invested in Ruby's success and have ample time for one-on-one training that is critical for becoming a skilled neuroscientist. Technical training will be under our supervision and also will involve others in my group including Eileen Nguyen (expertise targeting the RVM; **Letter of Support**), Kelly Smith (expertise in electrophysiology; **Letter of Support**) and Tayler Sheahan (expertise in behavior; **Letter of Support**). In addition, I will meet with Ruby for at least one hour per week to discuss recent data, relevant literature and plans for future experiments. Ruby will also present her research findings at our weekly lab

meetings (joint with the Koerber lab), thereby giving her practice in oral communication and organization of data into representative figures. This weekly meeting encourages the lab members to think broadly about other projects and to share their experiences.

2) Broad training in neuroscience and biology:

Intramural Interactions: Beyond her mentored research experience, Ruby will take a broad range of courses including statistics, ethics, and two pain-specific classes (Mechanisms of Pain taught by Michael Gold, and Pain Models, taught by me). She will attend seminars (~1/week), including 12 Pain Center Seminars, as well as a wide array of other neuroscience seminars that are advertised through the Pittsburgh Brain Institute. Ruby will regularly have the opportunity to meet with and even act as a host for seminar speakers of particular interest to her. She will present her research in several different formats to different audiences on an annual basis. These include a work-in-progress presentation to the Pain Center, a poster at Brain Day, a poster at the MSTP retreat, and a short-talk at an annual 3-day CNUP (graduate program) retreat. Ruby will also participate in the Pain Journal Club, which meets weekly to discuss a paper.

Extramural Interactions: Part of Ruby's training entails attending at least one extramural conference a year in order to diversify her professional and scientific exposures. I let trainees pick which meeting they would like to attend. Next year, she may wish to attend the Keystone meeting (there are concurrent meetings on Pain and Somatosensation), which I have helped organize, or the International Association for the Study of Pain (IASP), which will be held in Amsterdam. I like to bring trainees to meeting that I attend so that I can introduce them to my network of colleagues. I also encourage all of my trainees to apply for the North American Pain School (NAPS, a week-long boot camp), which is an outstanding experience.

3) Professional Development

Ethics: Ruby will participate in the bimonthly Clinical and Translational Science Institute's "Responsible Conduct of Research" (RCR) workshops that include lectures by experience faculty and case study-centered group discussions on multiple aspects of research ethics. These workshops provide trainees at all levels with an opportunity to interact and discuss issues such as (but not limited to) mentoring, research involving animal and human subjects, managing conflict of interest, and publication. We take training of our students in the ethical conduct of research seriously and frequently raise relevant issues at group meetings, as well as during one-on-one interactions with lab members.

Clinical: Ruby will participate in two LCC rotations (20-week clinical rotations for one half-day per week) to provide 1) direct experience balancing both clinical and scientific work and 2) develop valuable clinical skills in Ruby's field of interest, anesthesiology, during a scientifically rigorous part of her overall training. The LCC will provide Ruby with the clinical context for her science research as she begins to think about integrating her research and clinical work, a crucial aspect of her planned career as a physician scientist performing translational research. Ruby will also have frequent professional development meetings with Ajay Wasan **(Letter of Support)**, who combines an active research program and clinical practice as the director of the Pain Clinic. As an academic anesthesiologist, Dr. Wasan will be a fantastic role model and mentor for Ruby.

Communication in science: Through the Professional Development courses offered by the MSTP, Ruby has received formal instruction on scientific analysis of literature, writing and presenting, and networking skills. Ruby will actively gain oral communication experiences through lab meetings, journal clubs, poster presentations at local (CNUP and MSTP retreats) and national/international meetings (e.g., SfN, and IASP), and work-in-progress meetings through the Pittsburgh Center for Pain Research Pain. She will also learn skills in networking and establishing collaborations at these extramural meetings. To ensure that Ruby actively develops her written communication skills, Ruby will be expected to write or contribute written work from the lab in the form of book chapters, review articles, or primary papers. Ruby will develop her knowledge of and critically analyze the scientific literature through her active participation in journal clubs as well as assisting me review manuscripts several times per year. Lastly, Ruby will receive experience assuming a teaching role to undergraduate students within the lab.

Feedback: Ruby will meet with me daily through informal, impromptu interactions to briefly update me on his experimental progress. Weekly meetings will entail overall research progress, data analysis and literature discussions. More importantly, Ruby will benefit from the more serious meetings that I will schedule with her several times per year to provide constructive feedback for her scientific development as a graduate student.

C.II Environment

Ross Laboratory*:* My personal philosophy is that graduate advisors demonstrate unwavering support for their trainees. Science offers the thrill of discovery. For students early in their careers, I create a supportive environment to keep students motivated when encountering hardships. I believe that inspiring students in this manner builds their confidence to pursue visionary experiments that lead to breakthrough discoveries.

Koerber Laboratory: With forty years of experience in graduate training, I provide steady guidance to students and endeavor to share my expertise with trainees so that they can develop into rigorous scientists.

PCPR: Ruby will receive outstanding training as a member of the Pittsburgh Center for Pain Research (PCPR). Our Pain Center is made up of eight core faculty along with their trainees. These faculty members, in addition to Rick and myself include; Michael Gold, Brian Davis, Kathy Albers, Rebecca Seal and Brad Taylor. Most of these investigators, including Rick and myself have contiguous space on the 14th floor of the Biomedical Sciences Tower (BST). Center faculty and trainees meet weekly for journal club and twice monthly for work-in-progress talks. Ruby will present in both of these forums every year. In addition, the PCPR has an outstanding monthly seminar series in which leaders in the field of pain are invited to speak, and Ruby will have lunch with each of these speakers. We also offer two popular graduate courses, *Mechanisms and Clinical Presentation of Pain* as well as *Pain Models: Rationale, Testing and Interpretation*, which Ruby will take. Finally, the PCPR faculty are collegial and highly interactive, which is fostered by being in contiguous space and virtually daily interactions, including an annual skating party and numerous happy hours. For more details about the PCPR and its activities, see *http://pcpr.pitt.edu.*

CNUP: Ruby will be completing her thesis within the Center for Neuroscience at the University of Pittsburgh (CNUP), a well-established graduate program with over 100 faculty and 80 students spanning 20 departments across The University of Pittsburgh and Carnegie Mellon. Activities include an annual three-day retreat in the fall as well as Brain Day (local retreat) in the spring.

Physical environment: The Koerber and Ross labs are on the same floor as 5 other members of the Pain Center. On the floor there is also a conference room, shared offices for trainees, and a kitchen/eating area, locker rooms with showers, and two large relaxation areas with outstanding views of the city (east and west). These spaces foster wellbeing and create an ideal environment for learning and scientific exchange. Every lunchtime can be an impromptu meeting or think-tank between scientists from all backgrounds and at all levels. Ruby regularly interacts with these investigators and their students and postdocs, gaining valuable feedback on her project as well as mentoring advice.

C.III Research Facilities

Ruby will have access to all the equipment and facilities necessary to carry out her project.

The **Ross lab** occupies approximately 1,000 sq. ft. of space on the 14th floor of the Biomedical Science Tower (BST). The lab owns two electrophysiology rigs fully equipped with the following: air table, upright microscopes (Olympus), CED power1401-3 interface with associated Signal 5 software, an Axoclamp 2B microelectrode clamp, AC & DC amplifier, Sutter or Scientifica micromanipulators, temperature control units, XM10-IR Olympus CCD camera, Lumencor SOLA LED light source. We have a Leica CM1950 cryostat, Leica vibratome, small animal sterotaxic, dissection hood, Leica dissecting microscope, an RNAscope FISH oven, and two epifluorescent microscopes, -20C and -80C freezers, tissue cell culture room and perfusion room. Shared equipment on our floor includes a Nikon A1R fast-scanning confocal microscope (housed in the Ross Lab), cold room, a warm room, cell culture facilities, sterilization apparatus, dry ice, liquid nitrogen, and ultracentrifuges.

The **Koerber lab** occupies 1200 sq. ft of space on the 14th floor of the Biomedical Science Tower.

Electrophysiology: The Koerber lab has 3 complete electrophysiology recording rigs with each one equipped with an isolation platform, an upright fluorescent microscope (2 Leicas, 1 Olympus), One rig has an Axon Instruments Digadata 1322A with Axoclamp software, An Olympus BX51WI upright fluorescent microscope with a Sutter Instr. DG-4 light source with computer controlled shutters and multiple cubes for emitting blue and green light for activation of channel Rhodopsin and Archrhodopsin and a Hamamatsu ORCA-ER camera. The others have CED mini 1401 digital interfaces with appropriate software including Spike and Signal, all have an Axopatch 200B or Axoclamp 2B electrometer, a Narishige or Siskiyou 4 axis micromanipulator, appropriate AC & DC amplifiers, A computer controlled 50mW blue laser (473 nM) (Laserglow, Toronto) for optogenetic activation of cutaneous afferents. Two Yale University thermal stimulator and controller. Two Aurora Scientific mechanical

stimulators controlled by DACs from the CED 1401s. Each setup also has oscilloscopes, computers and monitors. I also have two dissecting stations complete with Zeiss Stemi 2000 dissecting microscopes with digital cameras and monitors for instructional purposes.

Histology and molecular biology: Leica CM 3050 cryostat, a Micron sliding microtome, Lica DMR fluorescent microscope, a RITEGA 2000B digital camera and computer/ monitor and Q-imaging software. Bio-Rad CFX Connect Real-Time PCR System, Thermal cycler, vacufuge, refrigerated centrifuge, Polytron homogenizer, thermomixer, spectrometer, western blotting and PAGE gel apparatuses, Gel dryer.

Animal Facilities: Mice are housed in a recently constructed vivarium facility in Biomedical Science Towers 3 within the University of Pittsburgh Division of Laboratory Animal Resources (DLAR). The facility has state of the art caging systems, rooms for quarantine and for breeding specialized populations, and a suite for BSL-2 work. This facility is fully accredited by the American Association for Accreditation of Laboratory Animal Care and is connected by a bridge to the BST where the Ross laboratory is located. This facility is professionally staffed by a manager and animal care technicians who provide basic animal care and is overseen by a primary veterinarian, Dr. Beth Ahner, and a back-up veterinarian, Dr. Edwin Klein. The Ross lab maintains a large breeding colony (including the Oprm1-cre allele to be used in this study) that is maintained by a full-time staff member.

Animal Behavior Core Adjoining the animal housing area is an animal behavioral core with full-time support staff. This core facility contains all of the equipment for a very large number of pain behavioral assays including Hargreave's apparatus, Von Frey testing, and high-speed video cameras that are required for this proposal.

Computing: Ruby has her own personal desktop that is networked behind the University of Pittsburgh firewall and are supported by dedicated IT staff in the Department of Neurobiology.

D. Number of Fellows/Trainees to be Supervised During the Fellowship

Dr. Sarah Ross: The Ross lab has two graduate students (Catherine Ruff and Eileen Nguyen) and two post-doctoral fellows (Taylor Sheahan, and Kelly Smith), as well as a third postdoc (Charles Warwick), who works jointly in the Koerber and Ross labs.

Dr. H. Richard Koerber: The Koeber lab has one graduate student (Joseph Salsovik) and one postdoctoral fellow (Charles Warwick), who works jointly in the Koerber and Ross labs.

E. Applicant's Qualifications and Potential for a Research Career

Co-Sponsor: Sarah E. Ross, PhD.; Associate Professor of Neurobiology

Ruby is a truly exceptional student. I would easily rank her in the top 1% of students that I have seen at the University of Pittsburgh and at Harvard Medical School. She knows exactly where she wants to go in life and is very focused on achieving her goals. I am positive that Ruby is PI material, and I committed to giving her the training and opportunities that she needs to help launch her towards the goal of becoming a physician scientist.

Ruby worked for four years as an undergraduate and an additional year as a research technician with Bart De Jonghe and Matthew Hayes (University of Pennsylvania) studying the circuitry in the brainstem that mediates chemotherapy-induced nausea. Her work contributed to five peer-reviewed publications, including a first-author publication, as well as a study in *Cell* on the role of the parabrachial nucleus in suppressing hunger in the context of pain. As attested by her letters of recommendation, Ruby was one of the brightest and hardest working students at Penn.

Ruby selected the University of Pittsburgh for her MD-PhD training because of the strength of the Pittsburgh Center for Pain Research and her desire to study the brainstem circuitry that modulates pain. I was thrilled when she selected my lab for graduate training because I could feel the spark and passion that Ruby has for research.

In my lab, I encourage my trainees to work on whatever scientific problem they find most exciting and to develop their own thesis projects independently. Towards this end, Ruby and I met multiple times over this past school year while Ruby was completing her second year of medical school to discuss her ideas for a thesis project. Ruby is drawn to the circuitry of the RVM because of her familiarity with the brainstem. Through her previous research experiences and fruitful discussions with multiple members of my lab, Ruby developed an interest in MOR signaling in the RVM. The approaches detailed in Ruby's proposal were inspired by the preliminary work of Eileen Nguyen (a second-year MD-PhD student in my lab) who had made some exciting discoveries through her research on putative OFF-cells and developed surgical strategies to selectively target the RVM with precise stereotaxic injections. Just at this time, we had imported the Oprm1-cre allele (developed by the Palmiter lab)

for another project. When these factors aligned, Ruby recognized an opportunity to use this new genetic tool to advance the field of pain by studying MOR+ RVM spinal projections in the RVM (putative ON-cells), and selected this project for dissertation work. After meeting with me several times to discuss her aims, Ruby wrote the entire NRSA application on her own, and then made minor modifications based on feedback from Rick Koerber, Michael Gold, Bart De Jonghe, several trainees in my lab, and myself.

Since Ruby is an MSTP student, she has outlined experiments that she can accomplish within a 4-year framework. I am confident that Ruby will make rapid progress towards these aims because she is a very hard worker with a lot of experience and a history of highly productive endeavors, including numerous papers.

Rick and I are very excited about Ruby's proposal because it has all the features that are appealing to a mentor who wants to ensure success for their trainees: it is a very *safe project* with lots of *opportunity to learn* new approaches together with huge *potential for big discoveries*. The project is a feasible yet compelling learning opportunity because all of the experiments proposed therein, while challenging, involve techniques that are currently working in our labs — Eileen Nguyen is currently using AAVr strategies to retrogradely label RVM projection neurons that target the spinal cord and cannulas implanted into the RVM to deliver CNO **(Letter of Support)**; Kelly Smith, a postdoc in my lab with years of electrophysiology experience from Brett Graham's lab, is currently performing pharmacological analysis and post-hoc reconstruction of spinal cord neurons recorded in slice **(Letter of Support)**; finally, Taylor Sheahan, a postdoc with years of behavioral experience from Rob Gereau's lab is currently analyzing the behavioral consequences of manipulating neurons in the spinal cord **(Letter of Support)**. Thus, in addition to mentorship from me and Rick Koerber, she will also get direct, hands-on training as she learns an array of news skills including anatomy, physiology, and behavior.

Ruby's project is exciting in terms of its research potential because she is studying a very important population — putative ON-neurons — in a new way, using tools and strategies that will allow her to visualize (aim 1), manipulate in vitro (aim 2) and in vivo (aim 3) the RVM neurons that send descending projections to the spinal cord to facilitate pain. If her hypothesis is correct (confirming the current model of ON-cell modulation of pain), her experiments will have provided a new level of granularity to the underlying circuitry; if her hypothesis is incorrect (challenging the current model of ON-cell model), her discoveries have the potential to change textbook dogma, eventually leading us towards more accurate models of descending modulation.

I am delighted to provide mentorship to Ruby as she undertakes the experiments enumerated in this proposal and I am committed to her success. As evidence of this, even though I am still relatively junior, my mentorship of MSTP students has already been recognized by virtue of being short-listed for the *William E. Brown Outstanding MSTP Mentor Award*. Thus, I believe that with my guidance and support, Ruby has the potential to develop into a highly successful physician scientist who will have a positive impact on science, medicine, and the community around her. Ruby has all of the attributes, personal and professional, of a first-rate scientist and I give her my enthusiastic support for this NRSA award, which would provide her with the recognition that would help launch her career.

Co-Sponsor: H. Richard Koerber, PhD; Professor of Neurobiology

To characterize Ruby Holland as a whip-smart student and a diligent worker would be an understatement. As an undergraduate at Penn, she had a first author paper and contributed to four others. Her letters say it all: Ruby is outstanding.

Understanding the specific circuitry in the spinal cord that is modulated by ON-cells remains a major gap in the field. I believe that the experiments that Ruby has articulated in Aim 2 (which I will supervise) will begin to address this gap by identifying the cell type(s) that receive direct input from MOR-expressing RVM neurons that project to the dorsal horn and by characterizing this input through optogenetic approaches. With my 40+ years of expertise in electrophysiology and anatomy in the dorsal horn, I am extremely well-suited to supervise this aspect of her training.

I am delighted to co-sponsor Ruby for this proposal, which I find very interesting, and to provide her with mentorship. Already, Ruby has shown great potential for a successful career as an independent physician scientist and has surrounded herself with a mentoring team, including her physician-scientist mentor Dr. Ajay Wasan, and an environment with the proper expertise, support, and dedication to her success. Ruby is a highly meritorious candidate for this F-award.