

Equipment

General: The Ross Lab is well-equipped with multiple freezers (-20°C and -80°C), three centrifuges, two epifluorescent microscopes (an Olympus BX53 upright and a Nikon TS100 inverted), a Coolsnap camera, a Leica CM1950 cryostat, a Leica 1200 vibratome, a dissection hood, two Leica stereo dissecting microscopes (one for fresh and one for fixed tissue) a Leica EC3 camera (to watch and record dissections), and a backdraft table (for animal perfusions).

Stereotaxic and Spared Nerve Injury Surgery: Located in the vivarium is a surgical setup with stereotaxis (KOPF) for surgeries such as craniotomy and viral injection. Ear and bite bars are in place on this apparatus, as is a complete homeothermic blanket system. A stereoscope (Leica Microsystems M80, 6x) allows for magnification and visualization of surgical site. Surgical tools (Fine Science Tools), a dental drill for craniotomy (Foredam), surgical sterilization equipment (Dent-Eq glass bead sterilizer), and a modified microliter syringe-pump (Hamilton 700 series) for injections are located onsite.

Microscopes: The Ross Laboratory has access to a Nikon A1R resonance confocal microscope and NeuroLucida system for reconstructing cell morphology. Dr. Zachary Wills, a PI within the Department of Neurobiology, provides extensive training and assistance as well as maintenance for this equipment. We are very fortunate to have access to advice from Simon Watkins, who runs the Center for Biologic Imaging (CBI) at the University of Pittsburgh. The CBI currently employs four faculty members and 20 staff, including multiple post-doctoral fellows, students, and technicians. The CBI facility is connected to the same building as Dr. Ross's laboratory and has fully equipped microscopy suites, computer labs, as well as wet and dry bench space for light and electron microscopic preparations. There are over 19 confocal microscopes of different types, as well as multiple systems dedicated to super-resolution imaging. These are available to students after completion of training.

Behavior: For the acute and chronic pain assays, the Ross Laboratory has four Sony Handycam HDRXR260V high definition video cameras and a Panasonic BMP-BD85 Blu-ray player connected to a 27" monitor. These will be used to record and analyze pain behaviors. Behavioral experiments will be performed within the Rodent Behavioral Analysis Core (RBAC) within the university's DLAR vivarium. The RBAC gives access to open field boxes for recording spontaneous and capsaicin-induced pain behaviors, as well as a Hargreaves apparatus and Von Frey microfilaments.

Slice Electrophysiology and Optogenetic Stimulation: The Ross Lab owns two set-ups for *in vitro* brain slice experiments. Each setup includes an air table, upright microscopes (Olympus), a CED power1401-3 interface with associated Signal 5 software, an Axoclamp 2B microelectrode clamp, AC and DC amplifiers, Sutter and Scientifica micromanipulators, temperature control units, an XM10-IR Olympus CCD camera, a Lumencor SOLA LED light source, and a microelectrode puller (Sutter P2000). For the duration of the proposed award, I will have exclusive access to one of the electrophysiology setups. Additionally, the Ross Laboratory has facilities, equipment, and materials for preparing solutions, cutting spinal cord slices, and fabricating electrodes.