Human Tissue / Vertebrate Animals:

Use of human tissue:

All human tissue used will be obtained from sources that have broadly consented donors for use in this proposed study and were collected for uses other than this specific study. All proposed activities meet the criteria for human subjects exemption, as defined by the National Institutes of Health, as tissue will be de-identified prior to acquisition and all investigators will be blinded to the donors and will not be able to ascertain the identity of the donors. Investigators will not have access to personally identifiable information and will not generate additional identifiable genomic data.

Detailed description of the proposed use of animals:

Both male and female C57BL/6J or CD-1 IGS wildtype mice, ages E11 to P30, will be used for the proposed experiments to provide retinal tissue for the single cell analysis. These studies will require approximately 20 pregnant females and 30 male and female mice (newborn to adult). Experiments will be carried out on excised, and enzymatically dissociated retinal tissue. The tissue will be isolated and immediately processed as required for each specific assay. Prior to sacrifice, animals will be housed in the Johns Hopkins American Association of Laboratory Animal Care (AALAC) accredited animal facility.

Justification for the use of animals, choice of species and numbers of animals to be used: The major goal of this research is to describe the developmental organization of the mammalian retina across time and space using single cell measurements. Because of fundamental gaps in our knowledge of retina development, no effective computational models have been developed that could replace the experimentation with animals proposed here. Nor can the precise and complex interplay of activity in intact tissue organization be mimicked in cell culture or other *in vitro* systems. Because of the increasing sophistication of the genetic tools available in the mouse, mice have become a standard model organism for studying the function of neural circuits. Therefore, all the proposed experiments will be performed in wild type mice. The use of experimental animals has been carefully considered in the design of this proposal. We are restricting our investigations *in vivo* to questions where an *in vivo* investigation is critical. The number of animals assigned to each experiments represents the minimum that we expect to make possible statistical analysis and thus interpretation of results. This number takes into account that some animals may be needed for unanticipated validation experiments. Based on experience, we computed approximately 10% more mice than those necessary for statistical analysis. Every effort will be made to minimize the number of animals used for the described experiments.

Veterinary care: Mice will be housed in a barrier facility at Johns Hopkins (BRB), an AALAC accredited facility. All procedures will be performed in the BRB according to governmental and NIH requirements. Veterinary care is provided by the Research Animal Resources (RAR) at Johns Hopkins. JHU maintains the expectation that all veterinary care for JHU research animals will be provided by RAR veterinarians or with RAR veterinary guidance to ensure quality and contemporary standards of care. Veterinary care is available 24/7 via routine rounds and a rotating on-call schedule. Veterinary care is provided by 4 faculty veterinarians, and 6 veterinary fellows. Surgical and technical support is provided by RAR rodent technical specialists. Daily husbandry procedures are provided by trained animal technicians. The veterinarians work directly with the animal care staff on programs designed to reduce the prevalence of infectious disease, the monitoring of animal health, and the diagnosis and treatment of illness and disease. Veterinary staff reserves the right to intervene in all cases in which animals are experiencing unalleviated pain or distress that has not been justified in the protocol as necessary to accomplish scientific objectives and for which provisions for palliative care have not been provided. An animal protocol, which is reviewed annually, has been approved for these studies by the Institutional Animal Care and Use Committee at Johns Hopkins

Procedure to minimize pain and discomfort: Every effort will be made to minimize any discomfort or distress to the animals being used. The surgical procedures are relatively straightforward and will be performed after extensive training and under supervision by experienced laboratory personnel and RAR veterinary staff. Mice will be carefully monitored for signs of distress as they recover from anesthesia and in the days following the surgery. If there is any sign of distress, animals will be euthanized immediately.

Euthanasia: Animals are inspected daily by the veterinary care support staff. Any moribund animals are noted and treated as appropriate (hydrogel addition, saline injection) and monitored closely over the next 24 hours. Those animals not responding to treatment will be euthanized. Euthanasia in all instances will be terminal inhalation of carbon dioxide or isoflurane vapor, followed by secondary euthanasia through cervical dislocation or decapitation. Methods of euthanasia are consistent with the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia, and in accordance with protocols approved by the Animal Care and Use Committee at Johns Hopkins University.