Characterization of an Unusual Collection of Olfactory Neurons in the Nose

Thesis by

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To borrow loosely from Hillary Clinton, it takes a village to educate and train a young scientist. I am indebted to so many people for their support, advice, and patience during my time here at Caltech. It is impossible to list them all; the people I will mention below are only a small subset of those who have positively impacted me during my graduate career. So much time in doing science is spent measuring things (milliliters in a tube, cells in a ganglion, years in graduate school), but the inspiration and strength lent to me by those who populate my life are immeasurable quantities, indiscrete and unbounded. Thank you to you all.

I have always assumed that the continuous financial and scientific support provided to my project by my thesis advisor, Scott Fraser, is a vote of confidence in my abilities as a young scientist. I know that it has not always been easy for him to give this support in an era of shrinking budgets and expanding time commitments. I am so appreciative and thankful for the sacrifices Scott has made for me. Scott often has good ideas. In our discussions, he is always upbeat, and he has helped me weather the inevitable scientific ups and downs that I have encountered in my time here. Scott also has a great sense of humor. One of my regrets is that I will likely leave Caltech having never pulled off a great prank on him. Oh well, there is always time. One can never get too comfortable.

I am indebted to David Koos, my partner in crime (crime: GG-ology), field general of the GG project, “the nasal fox.” A significant argument can be made that the GG should actually be referred to as the “Koos ganglion” (KG). Cosmically, the neurons would have then expressed pGC-K instead of pGC-G, which would have been either a great boon to my career (a novel guanylate cyclase isoform!) or a detriment (no antibody, no discovery). When I began my rotation in the Fraser lab in 2006, David already knew how to dissect and image the GG, a great starting advantage for me. David has helped me out in almost every imaginable way (only an exaggeration because I have a powerful imagination) in tackling a daunting project, whether it was with doing experiments

in a hands-on way, scheming the next experiments, providing encouragement at the right times, being an advocate for my career, and dealing with the paperwork and grind of working with animals. David and I have had many long conversations about the GG and other, more-personal topics. I am grateful that he and I have had a chance to walk down the same road.

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All of the electrophysiology was performed on Cheng Xiao's rig. Not only did Cheng teach me how to patch clamp, he might actually be the first person in history to have obtained whole-cell recordings from GG neurons. Cheng: you are the man; I hope you still had enough time on your rig. Cheng was (and still is) kind enough to let me use his rig for many more days and much more time than either of us initially anticipated. One of these days, when I have more cash, I should probably buy him a micromanipulator. As rig captain, Cheng allowed me to worry about tissue collection and patching. I never had to spend weeks taking the rig apart to fix 60 Hz noise; Cheng would do this for me. In some ways, I regret not having true knowledge mastery of a rig, but I suppose one cannot be an expert at everything, though perhaps I am still looking to be an expert at something. Cheng also put up with more stupid questions from an electrophysiology novice (me) than he will probably hear for the rest of his life. But, somehow, he was always kind to me! Now that is a quality I would like to possess.

My other thesis committee members have provided great professional advice to me over the years. It was Paul Patterson's idea to puff GG neurons with cortisol. John Allman proofread my first paper. Paul has offered to lend me his equipment and expertise in the behavioral analysis of mice

with defective GG organs. John offered to teach me how to section large specimens and to extract RNA from small samples, with the hope that I could do cross-species comparisons of the GG and analyze the GG transcriptome. Given the time, I would have liked to have done all these experiments. It was wonderful to have so many options and directions in which to take this project. I have no regrets about patch clamping, but if it had not worked, there were many other experiments that could have been done. Knowing this reduced my stress level significantly.

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and a good strategy. I would also like to thank my little sister, Beringia Liu, for being supportive and not being outwardly embarrassed by her nerd brother. There are no words that can adequately express my gratitude to my family.

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ABSTRACT

We have used a combination of histochemical, electrophysiological, and behavioral approaches to study signal transduction, membrane biophysics, and chemosensory function in the neurons of the mouse Grueneberg ganglion (GG) olfactory subsystem. The GG is a recently appreciated collection of ~1,000 clustered primary olfactory neurons located at the anterior tip of the mammalian nasal cavity. Despite their far-forward position, GG neurons are fully trapped beneath a keratinized epithelium and are wrapped by glial cells. This raises the question of how they contribute to the sense of smell. We found that GG neurons have key components of cGMP signal transduction pathway and are molecularly similar to GC-D neurons, which project to the enigmatic necklace glomeruli in the olfactory bulb. In electrophysiological analyses, individual GG neurons spontaneously discharged action potentials in one of three distinct temporal patterns that were stable for >20 min. An auxiliary fast-inactivating Na^+ current accounted for the various discharge patterns in computer simulations of the neuronal ionic currents. Despite differences in baseline activity, the majority of GG neurons responded to specific mammalian pheromones. In behavioral experiments, we found that the weaning of adolescent mice induced GG activity; however, the effects did not depend on ambient temperature or the presence of other animals. Because GG neurons reside on a dense vascular bed, have specialized access to serum contents, and directly responded to pressure ejections of serum, their activity can likely be modulated by internally circulating hormones or proteins associated with specific physiological states such as stress. Taken together, our results demonstrate unusual molecular and functional aspects of a morphologically and anatomically atypical olfactory nerve.

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