--- Chapter 6 ---

Future directions

Taken together, the presented results demonstrate multimodal functions for GG neurons. GG neurons detect cues associated with parental separation in young mice in a CNGA3-dependent manner. These cues may be sourced from serum. Other operating modalities for the GG include the CNGA3-independent excitatory detection of DMP pheromones and the inhibitory detection of a broad set of odorants. These various chemosensory modalities complement previously-identified responses of GG neurons to alarm pheromone and cold. Understanding how these different functions are integrated with one another into the molecular, electrophysiological, and behavioral characteristics of the GG will be one of many continuing challenges.

Further experimentation will be needed to build on the circumstantial evidence that supports the "internal sensing" hypothesis. It is not known if the electrophysiological responses to serum and cortisol are affected by the loss of CNGA3. CNGA3-dependent responses would suggest that the serum used in patch clamp experiments contains the compounds that might have altered GG activity *in vivo*. It will be necessary to characterize the electrophysiological responses (if any) of the GG to serum from mice exposed to the full complement of the behavioral manipulations described in Chapter 5. Column purification with mass spectrometry may help identify the serum components that trigger the electrophysiological responses. Finally, it will be important to determine if removal of adrenal glands or the functions of other sensory systems (*i.e.*, through naris occlusion) impacts GG activity.

A second direction points to the outputs of the GG. GG neurons mediate freezing behavior in adult mice exposed to a putative alarm pheromone. This shows that the GG plays an important role in how animals respond to stress. Yet nothing is known about the mechanisms by which the necklace-like

glomeruli and their downstream innervation targets generate the fear behaviors. While acetylcholinesterase positivity in the necklace glomeruli suggests integration into brain cholinergic systems, we also found that dopamine β-hydoxylase (DβH) was expressed in the GG (Chapter 4). This result confounds a simple classification of the GG as an exclusive part of any one brain system. Previous work also supports noradrenergic innervation of the necklace-like glomeruli (Brinon et al. 2001).

Despite exhibiting uniform responses to their defined chemical and thermal stimuli, GG neurons project to form 8-12 glomeruli, suggesting a heterogeneous mapping of outputs to higher-order brain systems. The molecular and functional basis for the formation of multiple glomeruli has not been elucidated. Chemical detection by pGC-G and pGC-A on a background of heterogeneous spontaneous firing patterns may account for some of the apparent output heterogeneity. This model can account for 2 types of responses on 3 spontaneous firing patterns, giving a maximum of 12 glomeruli if each unique output permutation is represented by 2 glomeruli. An attractive alternate hypothesis is that the glomeruli serve as representative targets of a topographic organization of the GG organ. For example, each glomerulus may be the destination of axons projected from a specific cluster or set of clusters of GG neurons. Injections of tract-tracing fluorescent dyes (Kobbert et al. 2000), selective photoconversion of fluorescent proteins (Chudakov et al. 2007), and trans-synaptic tracing with viral (Miyamichi et al. 2011) or plant (Baker and Spencer 1986) proteins will help to evaluate these possibilities.

Because the necklace glomeruli are situated between the glomeruli of the MOB and AOB, the GG might modulate the outputs of the other primary olfactory subsystems. For example, GG activity may alter the sensitivity or acuity of the olfactory sense. This may be important for specific situations of stress, weaning, and exposure to cold or odorant-rich/odorant-poor environments, when an increase or decrease of odor discrimination faculties would be beneficial for survival. Studies of olfactory function with genetic and surgical ablations of the GG should begin to address this hypothesis.

On a cellular level, it would be good to know the ligands of the pGC-G orphan receptor guanylate cyclase. Discovering these ligands should be part of a larger effort to understand the signaling and transduction mechanisms of GG neurons. A goal is to replicate the heterogeneous patterns of

spontaneous firing in the GG using *in silico* models of their ionic conductances. To arrive at these models, molecular identification of the conductances will be important, so that transgenesis techniques can be used to isolate the individual ionic contributions. This research in the complex-systems aspects of the GG may yield novel approaches to modify patterns and rates of neuronal activity. Furthermore, due to its far-forward location, the ganglion should be amenable to the application of exogenous small molecules. Studies of the GG may ultimately reveal new approaches to alter physiology and behavior in mammalian species.

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