Gain Control and Sparse Representations in the Olfactory System of the Locust and Fly

Thesis by
Maria Papadopoulou

In Partial Fulfillment of the Requirements
for the Degree of
Doctor of Philosophy

California Institute of Technology
Pasadena, California

2010
(Defended March 17th, 2010)

Στον αξιολατρευτο μπαμπα μου και στην αγαπημενη μου μαμα

Acknowledgements

I feel forever grateful to many people at Caltech, friends and family for helping me navigate through this long process. They made it a very valuable and often very enjoyable learning experience.

First, I would like to thank my advisor, Gilles Laurent for having me in his lab and giving me the opportunity to be part of the very exciting research he has started. I especially want to thank him for giving me such an interesting project and the freedom to develop it. I feel very privileged to have been part of the lab that he has created. The people who made up the lab were very supportive and instrumental in my scientific training and thinking as a scientist.

I would also like to thank my committee members Erin Schuman, Thanos Siapas, Mark Konishi, and David Anderson for their useful feedback, patience through all my committee presentations and for comments on my thesis. I would particularly like to thank Thanos Siapas, whose advice I have sought outside of those meetings and whose encouragement during some hard personal times at the end of my Ph.D. was extremely helpful.

The Laurent lab has been an amazing place to work. I had the opportunity to interact with some great scientists who were also very generous people. First, I would like to thank Stijn Cassenaer for among many things, his advice, help and continuous support throughout this work. Also, I would like to thank him for

demonstrating how fun science can be and for teaching me to enjoy every moment in and out of the lab. In addition to being a great collaborator, I am thankful for providing me access to his setup, a step that was critical for performing some of the work described in this thesis. For the fly work, I am especially grateful to Vivek Jayaraman, Glenn Turner and Greg Jefferis, without whom the fly project would have never started. In particular, I am thankful to Greg for sharing the dGGN image of the single MARCM clone of the GH146 line. Vivek has always been very encouraging, enjoyable to discuss science with and has helped me keep a healthy perspective throughout graduate school while also teaching me the importance of assumptions. Whether hunting snails, aplysia or flies, they have all been very enjoyable scientific collaborations. I am also thankful to Glenn for always being very generous with his time and advice and for demonstrating the real meaning of experimental thoroughness and skating. I would like to thank Anusha Narayan for being an incredible friend, colleague and collaborator on many non-scientific projects through the years and also for making life so much more interesting, unpredictable and fun. Together with Cindy they made the basement of the BI feel like home. I would also like to thank Cindy Chiu for demonstrating the perfect rig setup, for gps, valuable culinary advice and for sharing the BI basement. I would also like to thank Ofer Mazor for many fun and educational experiences associated with TAing the electrophysiology class during my first year in graduate school. Together with Stijn, he made learning physiology extremely enjoyable.

I would also like to thank the following people: Ben Rubin for teaching me the art of bobbyfying one's rig and contemplating the many facets of any issue. Stephen Huston for teaching me that self-deprecation has no limits and Sina Tootoonian for his bright presence in the lab and the importance of chicken thighs in life. Mala

Murthy for help with fly work and Kai for welcoming self improvement comments. Mikko for the importance of temperature regulation in Greek / Finish rig room sharing situations.

Outside the lab I am thankful to Anne Hergarden and Jasper Simon who together with Vivek Jayaraman constituted our fly journal club, where we spent hours over lunch having animated discussions on papers, techniques and our research projects. We certainly found many uses for the paper tablecloth in Citrus Bistro.

I would like to thank the Siapas Lab and in particular Eugene Lubenov and Casimir Wierzynski for very useful feedback during my defense preparations, and in general for very inspiring science.

Melanie Pribisko Yen for her friendship and help on many projects, including the time she synthesized a compound for me that induces aggression in a particular ant species. This experience really brought home to me how privileged I was to be at Caltech.

I would also like to thank a support group of friends whom I met on my first day at Caltech. We started graduate school together and have been friends ever since: Lily Kharevych, Sean Gordon, Rogier Braakaman, Ivan Bermejo, Aaron Noel, Waheb Bishara and Daniel Chung. While all in different divisions, we shared common goals and they provided the crucial mix of encouragement and distraction necessary to survive graduate school. Over the years our group grew to include other friends/family: Eric, Marta, Raquel, Giselle, and Roshan whose company I have enjoyed greatly throughout the years.

While it is hard to recall any memories before being in graduate school, there are two longtime friends of 13 and 20 years, respectively: Samia Qader and Konstantina Stergiani whom I would like to thank for keeping me sane through the years.

Finally, I would like to thank my family. It is fair to say that I would not have been here without the many sacrifices my parents have made over the years, before and after arriving in the US. I am forever indebted to them for this and for the values they instilled in me; like pursuing a lucrative career, for example. I would also like to thank my new Belgian family for all their love. I am also grateful to my two amazing siblings for being such great role models that I can only hope to one day resemble. Specifically, I would like to thank my brother for a continuous stream of advice, encouragement and unconditional love and my sister for her unyielding love and support and for the wonderful person she is. Finally I am thankful to my 4-year old niece Sofia for playing with me and putting things in perspective.

Abstract

The giant GABAergic neuron (GGN) is a single, paired, non-spiking neuron that arborizes extensively in the mushroom body (MB) of the locust (Leitch and Laurent, 1996), where it overlaps with the dendrites and the axons of Kenyon cells (KCs). KCs are the intrinsic neurons of the MB and are thought to be important for olfactory learning and memory(Davis, 2004). We are interested in understanding the function of GGN in olfactory processing: in particular, its pattern of arborization makes it an attractive candidate for controlling or modulating KC responses to odors, with potential implications for learning and recall. Physiological recordings of KCs in the locust show that these neurons respond sparsely to odors, in marked contrast to their excitatory inputs from the antennal lobe (projection neurons or PNs) (Laurent and Naraghi, 1994; Perez-Orive et al., 2002; Stopfer et al., 2003; Mazor and Laurent, 2005). Inhibition appears to be critical to control KC response threshold, probability and duration during odor stimulation (Perez-Orive et al., 2002). We show that there exists a feedback loop whereby KCs provide excitatory input to GGN , which in return provides inhibitory control of KC excitability. We further demonstrate that manipulating GGN during olfactory stimulation affects odor-evoked subthreshold oscillations, as measured in individual intracellularly recorded KCs, or by a more global measurement of the local field potential. We also assess the influence of GGN by recording from a population of extrinsic MB

neurons that receive input from KCs in the β-lobe of the MB (β-LNs). We show that GGN can suppress KC activity to such an extent as to eliminate all spiking in the down-stream neurons. With these experiments in the locust, we show that GGN controls the gain of PN-to-KC information transfer and normalizes the output of the KC-population, much reducing its dependence on input strength.

With experiments in *Drosophila Melanogaster* we try to extend the generality of GGN as a solution for gain control in the MB. Specifically, we carry out intracellular recordings from a *Drosophila* neuron discovered by Greg Jefferis, which has extensive arborizations throughout the MB calyx and lobes, resembling the locust GGN. We show that this cell is GABAergic, that it is a non-spiking neuron, and that its response to olfactory stimulation is very similar to that of the locust GGN, including a graded response to increasing odor concentration.

Contents

Acknowledgements **iv**

Abstract **viii**

Table of Contents **x**

List of Figures **xii**

1 Chapter 1: Introduction **1**

1.1 Olfactory system 1
1.1.1 Characteristics of Olfaction 1
1.1.2 Olfactory Receptor & Olfactory Receptor Neurons 3
1.1.3 ORN postsynaptic targets 5
1.1.4 Kenyon Cells 11

1.2 Mushroom Body 14
1.2.1 Anatomy 15
1.2.2 MB's role in olfactory learning and memory 20
1.2.3 Physiology 22

2 A Single GABA-ergic Neuron Subserving Normalization for Sparse Encoding in an Olfactory Network **24**

2.1 Introduction 24

2.2 Results 27
2.2.1 KC input to GGN 29
2.2.2 GGN output onto KCs 37
2.2.3 Manipulation of GGN state during odor stimulation 39

2.3 Discussion 47

2.4 Methods 49
2.4.1 Preparation and stimuli 49
2.4.2 Odor Delivery 50

2.4.3 Intracellular recordings 50
2.4.4 Local Field Potential Recordings 51
2.4.5 Electrical stimulation 52
2.4.6 Immunocytochemistry 52

3 *Drosophila* GGN 54

3.1 Introduction 54

3.2 Results & Discussion 56
3.2.1 What makes this neuron a homologue of the locust GGN? 59
3.2.2 Generating a specific GGN line 65

3.3 Future experiments: 66
3.3.1 Closing the feedback loop; GGN output on KCs during odor 66
3.3.2 Behavioral assessment of GGN's gain control in olfaction 66

3.4 Comparison with recently published result 68

3.5 Methods 72
3.5.1 Fly lines 72
3.5.2 Fly preparation 72
3.5.3 Odor delivery 73
3.5.4 Electrophysiology 73
3.5.5 Immunohistochemistry 74

4 Additional GGN Features 75

4.1 Introduction 75

4.2 Results & Discussion 76
4.2.1 Facilitation 78
4.2.2 Lack of Direct Antennal lobe input in GGN 81
4.2.3 Assessing KC output through β lobe LFP recordings 82
4.2.4 GGN Ablation 84

4.3 Methods 88
4.3.1 Preparation and stimuli 88
4.3.2 Odor Delivery 89
4.3.3 GGN Intracellular recordings 89
4.3.4 Local Field Potential Recordings 89
4.3.5 KC Electrical stimulation 90
4.3.6 AL Electrical stimulation 90

5 Conclusions & Future directions 91

5.1 Future directions: 93
5.1.1 Locust 94
5.1.2 Drosophila 96

Bibliography **97**

List of Figures

1.1	Olfactory Bulb and Antennal Lobe Circuitry	6
1.2	Locust Olfactory Anatomy	9
1.3	Mushroom Body Anatomy	17
2.1	GGN Morphology	28
2.2	GGN & LFP Different Odor Concentrations	29
2.3	GGN Response to Different Odors	31
2.4	Excitatory Input to GGN	32
2.5	DC shift	34
2.6	Source of Inhibitory Input to GGN	35
2.7	GGN output onto KCs	39
2.8	Summary data for multiple experiments.	41
2.9	Effect of manipulating GGN activity on KC activity during odor stimulation	43
2.10	Effect of manipulating GGN activity on KC activity during odor stimulation	45
3.1	Drosophila olfactory system	57
3.2	Anatomy or Presumed GGN in drosophila	58
3.3	Drosophila GGN is GABAergic	60
3.4	Drosophila GGN Odor Response	62
3.5	Drosophila GGN Odor Response to Increased Odor Concentration	63
3.6	Output Synapses in GH146-Gal4/ UAS SpH flies	64
3.7	Additional GABAergic labeled GH146-Gal4 cells	71
4.1	Kinetics of GGN's response to KC & Odor Stimulation in 3 locations along GGN.	77
4.2	Facilitation of the KC to GGN synapse	78
4.3	Facilitation dependence on IPI.	80
4.4	GGN Response to AL stimulation.	82
4.5	β lobe LFP Increases with an Increase in Odor Concentration.	83
4.6	Enhancing GGN activity during Odor decreases LFP power in the & β lobe	85
4.7	Effect of ablating GGN on LFP power	86

Chapter 1

Chapter 1: Introduction

1.1 Olfactory system

1.1.1 Characteristics of Olfaction

Sensory systems have evolved to detect and process information about the external world with the task of extracting useful relationships for the organism's survival and procreation. The sense of olfaction in particular is integral in food and mate localization, as well as forming associations predictive of food (reward) and danger (punishment).

The olfactory system has evolved to detect small volatile molecules -odors- in the environment. Odors can be comprised of one component (termed monomolecular) but more often than not, odors are mixtures of many components, sometimes in the hundreds or thousands. The characteristic smell of a rose for example consists of about 260 components (Keller and Vosshall, 2004). Human psychophysics suggests that our brain has evolved to group and recognize such mixtures as a single odor, rather than identifying each of the mixture's components, suggesting that smell is a synthetic sense. In fact, such experiments show that human subjects at best can tell up to three or four components within a mixture. Interestingly, the odor of

mixtures is not perceived as more complex than the odor of single chemicals.(Keller and Vosshall, 2004).

Humans have the ability to generalize odors and to categorize them perceptually as fruity, citrus etc (Bitterman, 1983; Dravnieks, 1982). Bees have also been shown to generalize in behavioral assays. After pairing of an odor (unconditioned stimulus) with sucrose (conditioned stimulus), bees start to extend their proboscis to this odor alone in the absence of reward. They are able to identify the odor precisely, and they are also able to generalize to structurally similar odors (Smith, 1989). Therefore the olfactory system has evolved to extract both types of information with presumably the same machinery (Laurent, 2002).

Evolution appears to have found some optimal solutions for solving the problems discussed as demonstrated by how well the organization of the olfactory system is preserved across phyla and species (Strausfeld et al., 1998). I will briefly review the general organization and then expand in the following sections to explore in more detail the anatomy and the transformations that occur in each successive layer.

In both vertebrates and invertebrates odor molecules bind to specialized olfactory receptor proteins on the dendrites of olfactory receptor neurons (ORNs)(Shepherd, 1993). Upon odor binding an ORN is depolarized and transmits an action potential to the second relay in the brain, the olfactory bulb (OB) in vertebrates or the antennal lobe (AL) in insects. In particular, ORNs send axons into spherical neuropilar structures called glomeruli in the OB and AL where they contact processes of their postsynaptic partners. Only one class of such partners, mitral and tufted cells in the OB and the projection neurons (PNs) in the AL exit the second relay and target higher association areas. In rodents, those areas are cortical memory areas;

in insects, an analogous area associated with multimodal processing, learning and memory is called the mushroom body(MB).

Because of this organization, i.e., one layer (OB or AL) separating high association areas from the periphery, olfaction is considered a shallow sensory system. It appears, then, that the formatting of odor information in the second layer is sufficient before it targets learning and memory areas.

In summary olfaction is integral to the survival and procreation of many organisms, it is a synthetic and shallow sense, and the olfactory system has evolved to solve both identification and generalization problems. We next explore how this is achieved, starting with odor binding to the olfactory receptors.

1.1.2 Olfactory Receptor & Olfactory Receptor Neurons

Considerable advancement in olfaction research was made with the discovery and cloning by Axel and Buck of the first members of the odorant receptors proteins (Buck and Axel, 1991; Buck, 1996). Odorant receptors (ORs) are a large and diverse gene family of G-protein coupled receptors ($\sim$ 1000 genes in rodents, $\sim$ 350 in humans) (Wilson and Mainen, 2006; Keller and Vosshall, 2004)). There are many (est. 100-1000) unique olfactory receptors. Insects express a phylogenetically distinct family of olfactory receptors (Sato et al., 2008; Kaupp, 2010). In vertebrates these receptors are located in the cilia of the nose, while in insects along the length of the antennae (Mombaerts, 1999; Vosshall et al., 1999; Wilson and Mainen, 2006).

ORs become activated upon binding to an odor. Unlike the immune system where binding to a G-protein coupled receptor appears to be specific, ORs and consequently ORNs, appear to respond to a wide variety of stimuli. Even monomolecular odors drive broad ORN activation (Laurent, 1999), and studies of broad OB

lesions show that rats can still discriminate odors, pointing to a distributed activation of a combinatorial nature (Laurent, 1999). It appears therefore that an odor is encoded in the pattern of ORNs it activates (Hallem and Carlson, 2006). Furthermore, flies that express only one OR are still capable of odor discrimination, which has been interpreted to suggest that odors elicit different temporal patterns for a given receptor and that the temporal dynamics of the response could be an important coding variable(DasGupta and Waddell, 2008). In the locust AL, ORN responses to odor show temporal patterning that is both odor and ORN dependent. (Raman et al., 2010). The tuning of ORNs varies in breadth; there exist broadly tuned receptors as well as narrowly tuned ones, and many in between[1] (Su et al., 2009). As the concentration of an odor increases it leads to the recruitment of additional ORNs (Su et al., 2009).

While the focus so far has been on the *activation* of ORs by odor molecules, it is also possible for an odor to *inhibit* an ORN (decrease over baseline firing); this has been argued to be mediated via odor "antagonism" (Su et al., 2009). The duration of the ORN response is dependent on the receptor it expresses, as shown by recording of an ORN where the receptor expressed can be controlled genetically(Hallem and Carlson, 2004; Su et al., 2009).

Each ORN typically expresses one OR, which confers its sensitivity (Mombaerts et al., 1996; Vosshall et al., 2000). While olfactory receptor neurons are dispersed along the epithelium (rodents) and antennae (insects), the second layer appears to be more organized. ORNs target a glomerulus based on the OR they express; that is,

[1] It is worth mentioning that in addition to generalist channels there are some ORNs that respond to unusual odors (specialists), which are organism specific (pheromones) and the sensitivity and specificity to their ligand is often quite high. Upon pheromone binding to these specialist receptors, subsequent responses are highly stereotyped. Such examples include the CO2 receptor in insects (Suh et al., 2004) , and in the mosquito, an ORN that is highly tuned to a compound found in human sweat (Hallem and Carlson, 2004; Wilson and Mainen, 2006).

only ORNs with the same OR target the same glomerulus (Mombaerts et al., 1996; Vosshall et al., 2000). ORNs form excitatory synapses onto glutamatergic M/T cells in the OB. In insects ORNs synapse onto PNs, which are predominantly cholinergic.

1.1.3 ORN postsynaptic targets: Projection Neurons-Mitral/Tufted Cells & Local Interneurons

In addition to receiving input from ORNs, processes of M/T or PNs also contact cells locally within the OB and AL. Lateral connections within the OB as well as the AL can modify the input from the ORNs. In the OB, ORNs also synapse with periglomerular cells (PG), which are inhibitory, GABAergic, and, within a short range, extend to other glomeruli. PGs can synapse back onto the ORN axons to inhibit release, as well as onto M/T cells. M/T cells can excite each other laterally through spill over of NT from the apical tuft, but they can also inhibit each other via an intervening PG. M/T cells in addition activate granule cells that are axonless GABAergic cells residing in the external plexiform layer that in turn through dendrodendritic interactions inhibit M/T cells. The effects of these interactions can be long range as M/T cells extend dendrites 10-12 glomerular diameters, contacting many granule cells (Wilson and Mainen, 2006; Laurent et al., 2001)

In insects, similarly to vertebrates, ORNs excite both projection neurons and local inhibitory neurons (Wilson and Mainen, 2006; Laurent et al., 2001). In addition, PNs also target local neurons (LNs) (Leitch and Laurent, 1996). LNs are axonless neurons similarly to vertebrate granule cells, and can extend throughout the entire AL (Leitch and Laurent, 1996; MacLeod and Laurent, 1996; Wilson and Laurent,

2005). The majority of LNs are inhibitory, releasing GABA, but some are cholinergic, mediating lateral excitation (Shang et al., 2007; Olsen et al., 2007). The presence of this lateral excitation explains partly why PNs are more broadly tuned than their presynaptic ORNs (Wilson et al., 2004).

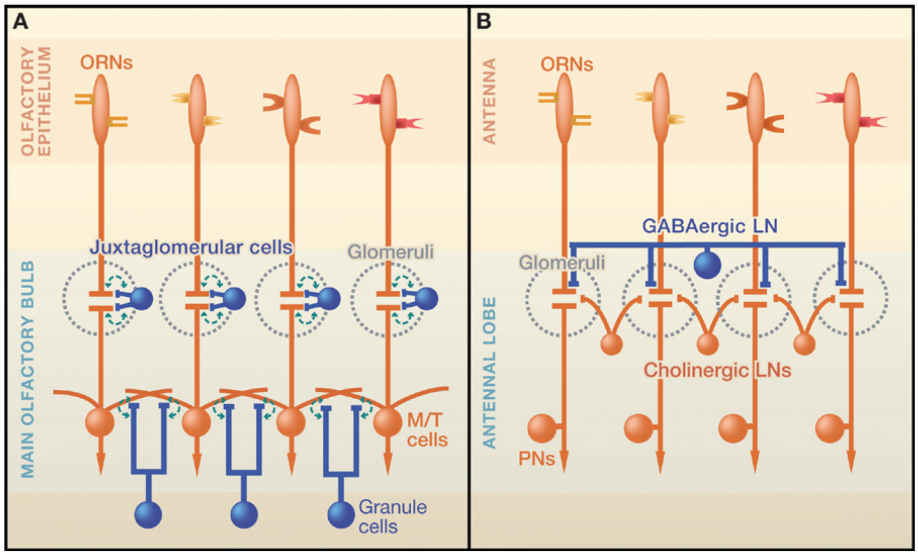


Figure 1.1. Olfactory Bulb and Antennal LobeCircuitry. Excitatory neurons are shown in orange and inhibitory neurons in blue. (A) Olfactory receptor neurons (ORNs) in the olfactory epithelium that express different olfactory receptors project axons to separate glomeruli (dashed outlines) in the olfactory bulb where they synapse on mitral and tufted (M/T) cells, whose apical dendrite is usually localized to a single glomerulus. Juxtaglomerular cells (blue) contribute to intraglomerular inhibition. In the glomerulus, ORNs form synapses on juxtaglomerular cell dendrites, which in turn inhibit ORN axon terminals. Reciprocal synapses are also found between juxtaglomerular cell and M/T cell dendrites. Reciprocal synapses are formed between the dendrites of granule cells and M/T cells. M/T cells excite granule cells, which respond by inhibiting M/T cells. Due to the lateral spread of M/T secondary dendrites, granule cells contact multiple M/T cells associated with different glomeruli, and thus can mediate both intra- and interglomerular inhibition. (B) In Drosophila, ORNs expressing the same olfactory receptors in the antenna or maxillary palp synapse on projection neurons in a single glomerulus, analogous to the olfactory bulb. GABA-releasing local neurons (LNs) presynaptically inhibit ORN axon terminals in multiple glomeruli, mediating interglomerular inhibition. Excitatory cholinergic LNs mediate interglomerular excitation. Figure from (Su et al., 2009)

A comparison of PNs and presynaptic ORNs in drosophila has shown that PNs respond more strongly and reliably; and odors can be classified more accurately based on PN than ORN responses. Such differences between the responses of PNs

and their presynaptic ORNs have been shown to arise partly because of the high convergence ratio of ORNs to PN and because the connection between ORNs and PNs is strong. Specifically, the convergence ratios of ORN to M/T or PNs are 5000:1 for rodents and ~ 100:1 in some insects . (Laurent, 1999; Wilson and Mainen, 2006). Such a high convergence ratio could serve multiple functions. By averaging it reduces noise and enhances the signal received by a M/T or PN. In particular, the spread of ORNs across the nasal epithelium and the antenna could serve to eliminate local fluctuations, thereby enhancing the signal and eliminating noise(Laurent, 1999).It could serve to also enhance the dynamic range of M/T provided that the thresholds for ORNs are different (Linster and Smith, 1999).

GAIN CONTROL IN THE ANTENNAL LOBE

There is a nonlinear amplification of ORN input to PNs, extending the dynamic range of PNs by amplifying weaker ORN responses more than stronger ORN responses (Olsen and Wilson, 2008). In this way, PNs can separate odors faster and better than ORNs(Bhandawat et al., 2007). Several mechanisms contribute to this. Firstly, reliability across the ORN-to-PN synapse, coupled with the high convergence ratios, ensures reliable PNs responses to weak ORN inputs (Bhandawat et al., 2007)). Secondly, the presence of short term depression between the ORN-PN synapse, ensures that gain of the synapse is turned low for strong inputs ensuring that PN responses remain within the dynamic range(Olsen and Wilson, 2008).

Lateral inhibition within the AL appears an important factor for adjusting the gain within a glomerulus, proportional to the total ORN activity (Olsen and Wilson, 2008). This lateral inhibition is provided by neurons in the AL that connect with most (if not all) glomeruli (Olsen and Wilson, 2008). While it appears that lateral

inhibition scales with total ORN input, there still exist differences across glomeruli with respect to total inhibition (Olsen and Wilson, 2008).

PROJECTION NEURONS

PN and MC responses have are distributed in time and space. That is, about 50% of all PNs in the AL respond to any odor, and their responses are often multi-phasic, containing bouts of excitation and inhibition lasting hundreds of milliseconds, generally outlasting the stimulus itself. These response patterns, are odor and PN specific (Wehr and Laurent, 1996; Wehr and Laurent, 1999; Laurent, 2000; Perez-Orive et al., 2002; Stopfer et al., 2003; Mazor and Laurent, 2005; Broome et al., 2006).

As mentioned above, LNs can be excited by PNs and in turn inhibit PNs and other LNs (Leitch and Laurent, 1996; MacLeod and Laurent, 1996). Upon odor exposure, the interaction between these two populations leads to the generation of 20-30Hz oscillations that can be recorded in the mushroom body(MB), the downstream area where PNs send their axons (Wehr and Laurent, 1996). These odor-evoked oscillations are generated in the AL; they are absent in ORN responses and they can be abolished by the application of a fast chloride channel blocker (picrotoxin) in the AL (MacLeod and Laurent, 1996; Wehr and Laurent, 1999). These oscillations can also be observed in LNs and PNs during intracellular recordings as subthreshold membrane oscillations. (Laurent and Davidowitz, 1994; MacLeod and Laurent, 1996; Laurent et al., 1996). The oscillations detected as local field potential (LFP) in the MB are the result from activity of a transient group of synchronized PNs. The identity of the active PN assembly changes during the odor presentation and PNs belonging to the ensemble fire both phase-locked and non-phase-locked action

potentials (Wehr and Laurent, 1996).

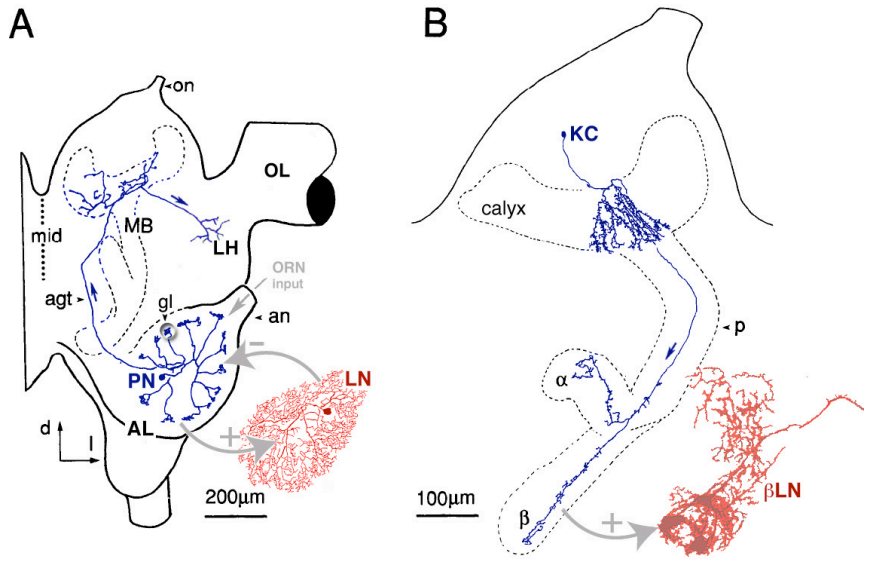


Figure 1.2. Locust Olfactory Anatomy. AL, antennal lobe; LH, lateral horn; MB, mushroom body; OL, optic lobe; agt, antennal-glomerular tract; an, antennal nerve; gl, glomerulus; on, ocellar nerve; p, pedunculus; βLN, β-lobe neuron; KC, Kenyon cell; LN, local neuron; ORN, olfactory receptor neuron; PN, projection neuron; d, dorsal; l, lateral; mid, midline. Adapted from Laurent and Naraghi (1994); MacLeod and Laurent (1996).

Oscillations and slow patterning have also been described in the olfactory bulb of mammal (Adrian, 1942; Adrian, 1950; Kauer, 1974; Hamilton and Kauer, 1989; Laurent et al., 2001) . Here we focus on results in locusts and in particular on studies that attempt to understand these phenomena and their contributions to olfactory coding. We also refer to some relevant results in zebrafish that further this understanding.

As already alluded to, PN responses to odor contain information at two prominent timescales. At a fast time scale (50ms) they fire spikes synchronously with other PNs and at longer timescale (hundreds of ms) they exhibit patterning of responses that reflect odor and PN identity (Wehr and Laurent, 1996; Wehr and Laurent, 1999; Laurent, 2000; Perez-Orive et al., 2002; Stopfer et al., 2003; Mazor and Laurent, 2005; Broome et al., 2006).

Experiments in the locust have shown that in order to understand how odors are encoded, it is important to consider the PN population activity as a whole and to extract information in a way that is consistent with how downstream cells decode it. In one such experiment, it was shown that when trying to determine how odor concentration is encoded in the AL by PNs, recording the response of a single PN to a range of odor concentrations does not provide much information. For example, one PN might increase its activity in response to a particular range of odor concentrations and then it might become inhibited at higher concentrations in a way that is not consistent across PNs (Stopfer et al., 2003). By recording simultaneously from 10-20 PNs and pooling across experiments, certain features emerge that characterize how these neurons encode an increase in odor concentration. As the concentration of an odor increases so does the extent of synchrony among PNs (Stopfer et al., 2003). Recordings from downstream neurons, the Kenyon Cells (KCs), have shown that the temporal integration of these neurons is effectively limited to a single LFP cycle or $\sim$ 50ms (Perez-Orive et al., 2002). If the activity of the PN population is represented as a vector, with dimensionality equal to the number of neurons, then the evolution of this vector, followed at 50ms time-steps, consists of a trajectory through PN activity space (Stopfer et al., 2003; Mazor and Laurent, 2005; Broome et al., 2006). Such trajectories reveal a family of curves for different concentrations that are distinguishable from each other, but still close together compared to sets of trajectories representing different odor families (Stopfer et al., 2003).

Collecting simultaneously recorded data and analyzing them as discussed has provided additional insights into how PNs encode odors. By examining odor pulses of different durations, it has been shown that these trajectories throuhg PN activity-space are optimally separated during the transient states of the stimulus, ie during

the onset and offset of the stimulus, rather than during the steady state (Mazor and Laurent, 2005). Recordings from the KCs downstream of the PNs have shown that these cells respond the least during steady state (Mazor and Laurent, 2005). Steady-state measures of activity thus seem less appropriate to understand the neural code in this system (Mazor and Laurent, 2005). Experiments that examine PN responses to two odors separated by different intervals show that for some delays, the state reached by PNs is different from the states representing individual components or from the mixture (Broome et al., 2006). These results suggest that the evolution of PN responses is very much history dependent. All these results point to time as a very relevant feature in olfactory coding. This is likely made possible by the relatively slow evolution of the odor stimulus, in comparison to visual or auditory stimuli.

One way in which time is thought to be used, is to extract/separate different features of the odor over time. Interaction within the AL and the OB leads to decorrelation; that is, responses across the population of PNs or M/Ts become more dissimilar over time (Laurent, 2002) . Through this processes, different aspects of the odor can be extracted at different time-points, which can then be passed on to the MB and processed for learning (Laurent, 2002). Evidence for decorrelation comes from recordings of M/T in zebrafish, which demonstrate that early responses are well-suited for categorization, while late response are more informative for stimulus identification (Friedrich and Laurent, 2001; Laurent, 2002).

1.1.4 Kenyon Cells

HOW ARE PN RESPONSES TRANSFORMED IN THE NEXT STAGE?

PNs carry olfactory information to the next layers of processing: to the mushroom body and to the lateral horn (LH). They send widespread projections throughout the calyx of the MB, where they contact Kenyon Cell dendrites (Jortner et al., 2007). Recordings from KCs have revealed extraordinary response transformations across the two layers; while PN odor responses are dense and exhibit prolonged slow patterning, KCs respond to odors rarely, and when they do, they only fire on average ~ 2.3 spikes/sec (Laurent and Naraghi, 1994; Perez-Orive et al., 2002; Stopfer et al., 2003; Perez-Orive et al., 2004). Kenyon cells responses are quite sparse, whether considering lifetime or population sparseness (Perez-Orive et al., 2002). Such sparseness is particularly attractive for a population of neurons involved in olfactory learning and memory. Several theoretical considerations point to the benefits of sparseness as a coding principle in terms of balancing storage capacity with minimizing overlap across representations and therefore the number of synaptic modifications required, as well as facilitating memory recall (Marr, 1969; Kanerva, 1988; Laurent, 2002; Olshausen and Field, 2004; Barlow, 1972; Field, 1994). It was recently discovered that KCs receive input from about 50% of all PNs. While this finding suggests that the number of potential patterns that can be encoded is very large, it also makes the KC sparseness all the more striking (Jortner et al., 2007).

Inhibition as well as intrinsic dendritic properties have been shown to underly this sparseness. One form of inhibition onto KCs has been identified to be feed-forward inhibition from a population of ~ 60 lateral horn interneurons, which themselves receive direct PN input (Perez-Orive et al., 2002). Because of the high

convergence of PNs onto LHIs, LHI output is oscillatory and responsive to all odors. Due to propagation and synaptic delays, the inhibition from LHIs is phase-lagged to the excitatory PN input by 180 degrees. As such it serves to reduce the KC integration window to ~ half an oscillation cycle (Perez-Orive et al., 2002). Additionally, voltage-gated channels in KCs amplify the effect of EPSPs that arrive in close temporal proximity and sharpen the EPSP. This also contributes to reducing the effective integration window, and making KCs highly responsive to coincident input (Perez-Orive et al., 2002)[2].

Considering these existing mechanisms, cell numbers and connectivity, Jortner *et al.* demonstrate that the response probability of a KC to a variety of inputs should change dramatically by adding only a small number of additional PNs (Jortner et al., 2007). This is not consistent, however, with experimental results. As mentioned previously, PNs respond to increasing odor concentration by an increase in synchrony. Since they act as coincidence detectors, KCs are very sensitive to synchrony among their inputs (Perez-Orive et al., 2002). And rather than a very steep and uniform increase in KC activity as a function of odor concentration, the recordings reveal that there are KCs that are concentration invariant, others that only respond to a limited range of odor concentrations and not at all to higher concentrations and so on(Stopfer et al., 2003). These data point to the existence of additional mechanisms to ensure KC sparseness. The main focus of this thesis, detailed in Chapter 2, consists of a form of inhibition that acts through a feedback loop to scale the activity of each KC by the total KC activity, thus dynamically

[2]Recent recordings from neurons in the piriform cortex, downstream from mitral cells of the OB, have shown these cells to fire sparsely to odors, similarly to KCs (Poo and Isaacson, 2009). Furthermore a recent study has shown a circuitry similarly to what we described for PNs-KCs-LHIs, where oscillatory excitation is odor specific and precedes inhibition that is global and broadly tuned. The combination of these two features reduces the integration window of pyramidal cells in the piriform cortex, contributing to their sparseness (Poo and Isaacson, 2009).

maintaining a sparse representation among KCs.

Consistent with the observation that the KC integration window is limited to half of one oscillation cycle, recordings from PNs and KCs under the same experimental conditions show that KCs decode PN output in a piecewise fashion, one LFP cycle at a time. As mentioned above, in experiments were two odors are presented with different temporal delays, for some delays, the PN population response (represented as an evolving trajectory) differs from either of the components or the mixture (Broome et al., 2006). Consistent with piecewise decoding from KCs, there exist KCs that selectively respond to a particular temporal delay and therefore to the PN ensemble that was activated but not to any of the components or the mixture(Broome et al., 2006).

In summary, KC responses are sparse and synthetic; KCs receive input from a large fraction of PNs, which they decode piecewise at each LFP cycle. KCs have a high threshold for activation, but when a large enough fraction of their inputs is activated they respond during the corresponding cycle with a rare action potential.

1.2 Mushroom Body

The mushroom body (MB) is a paired neuropil structure found in annelids and in all arthropod groups except crustaceans (Strausfeld et al., 1998). The mushroom bodies differ in size between taxa, as well as between different castes of a single species of social insect (Strausfeld et al., 1998). The MB was first Identified in 1850 by Felix Dujardin, who was the first to observe that there was a correlation between the size of the MB and the social complexity in different species of bees. That lead him to hypothesize that this area of the brain was not involved in simple reflex behaviors.

Lesion, genetic manipulation and behavioral experiments all point towards a role for the mushroom body in olfactory learning and memory(Heisenberg et al., 1985; de Belle and Heisenberg, 1994; Dubnau et al., 2001; Krashes et al., 2007; McGuire et al., 2001). Physiological recordings from the intrinsic cells in the MB have shown their responses to odors to be sparse (Laurent and Naraghi, 1994; Perez-Orive et al., 2002; Turner et al., 2008), a feature that could be beneficial for memory storage and recall (Laurent, 2002). Furthermore, the synapses between these cells and a group of extrinsic MB neurons, have been shown to be plastic and affected by the presence of the neuromodulator octopamine ((Cassenaer and Laurent, 2007) (Cassenaer & Laurent in preparation).The MB has additionally been implicated in sleep and decision making (Joiner et al., 2006; Zhang et al., 2007b). Here we focus on olfactory learning in insects species where most of these studies have taken place, review the anatomy and some of the data from the studies pointing to an important role for this area in olfactory learning and memory.

1.2.1 Anatomy

There is one MB per hemisphere, each being a mirror image of the other. The MB is a neuropil structure resembling a mushroom in shape, occupying a big volume of the brain. Kenyon cells (KCs) are the intrinsic cells of the mushroom body. Their number per MB varies between 2,500 in *drosophila* to 200.000 in the cockroach (in the locust there are 50,000, while in the bee 170,000). In bees KCs constitute 35% of all the neurons in the bee brain (340,000 KCs out of 960,000) , while in *drosophila* 5% (5,000 out of 100,000)

The MB anatomically can be divided in 3 areas; the calyx, the pedunculus and the lobes. The calyx is a cup like neuropil structure where KCs receive input; as

such it contains KC dendrites and axons of postynaptic neurons to these KCs (ie PNs, LHIs etc). The KC cell bodies sit above and in some case also besides the calyces. A typical KC cell extends a neurite from the soma that gives rise to a dendrite and an axon. While the dendrite targets the calyx of the MB, the axon runs through the pedunculus to reach the lobes. The pedunculus is a bundle of such tightly packed parallel KCs axons that run through right before they bifurcate to send axons ending in a vertical and medial lobe (termed α and β lobes respectively). In the lobes, KCs make output synapses with extrinsic cells of the MB.

These general anatomical characteristics can differ depending on the species. Some insects lack calyces, while social insects like the bees and ants have two calyces per MB (Strausfeld et al., 1998). Similarly for the lobes, there exists some variation; In some insects , of which *drosophila* is an example, in addition to a vertical lobe that in *drosophila* it is comprised of the α/ α' lobes and the medial β, β' lobes, there is a third lobe called the γ.

Drosophila KCs can be classified in three morphological distinct classes, based on the lobes they target ,ie α/ β, α' and β' or γ KCs. Furthermore there exists a correlation between these three classes and their birth order as well as their soma position. Specifically, there are four neuron precursor cells (neuroblasts) that give rise to KCs and as more KCs are born the older ones are pushed in the periphery, forming concentric circles with the later-born KCs in the center. The γ neurons are born first followed by the the α' and β'. neurons and last are the α/ β neurons (Lee et al., 1999)(Lee and Luo 99). As such in the pedunculus one can observe concentric circles with the α/ β neurons are in the center of the bundle and the γ neurons in the periphery.

In cricket KCs, there is a relationship between calyx arbolization, birth order

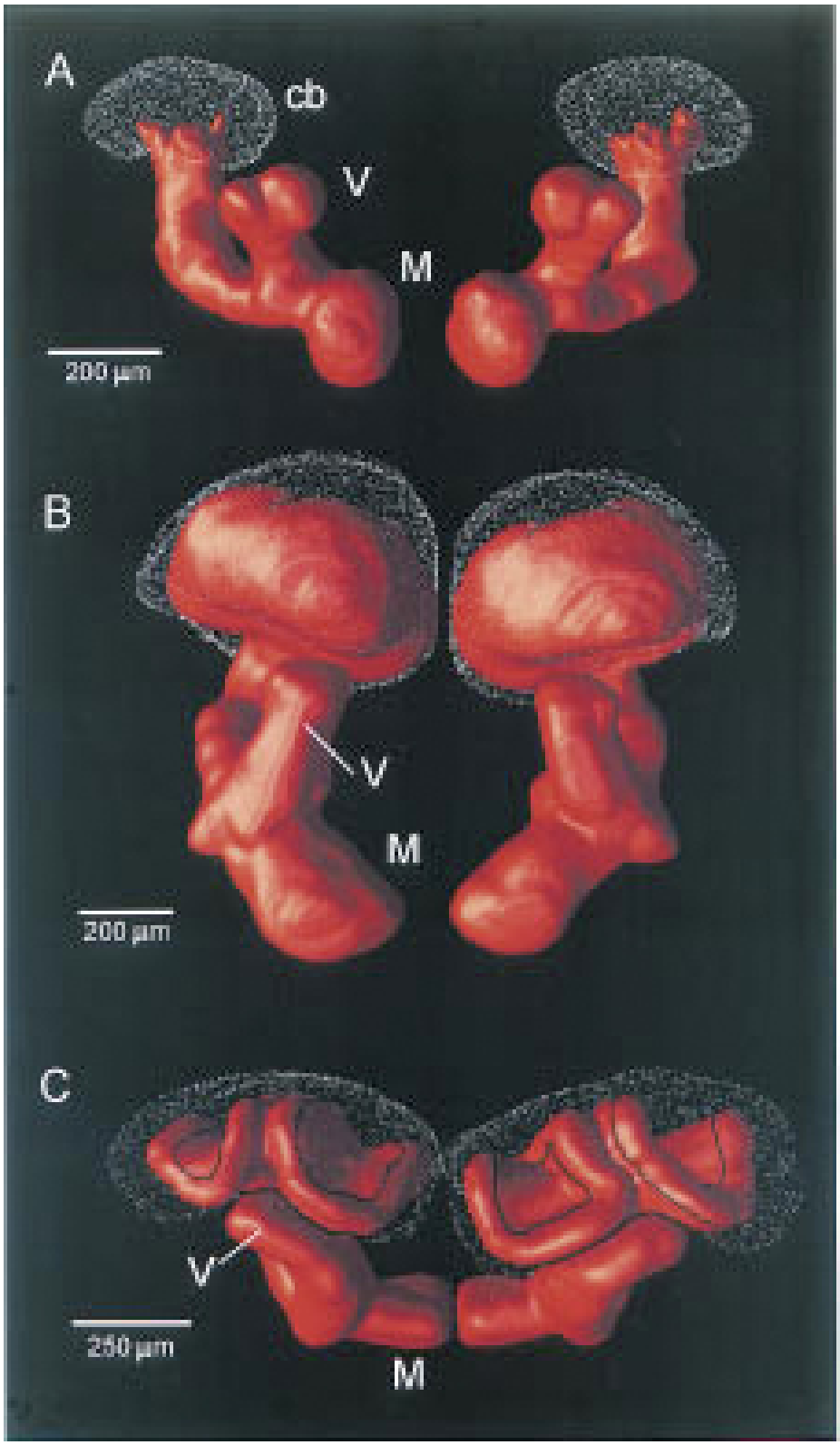


Figure 1.3. Surface-tessellated reconstructions of mushroom bodies. (A) Primitive calyxless condition in the silverfish Lepisma. (B) Single calyx in Schistocerca (locust). (C) Double calyces of the honeybee, Apis mellifera. (cb) Globuli cell bodies; (V,M) vertical and medial lobes, respectively. Figure from (Strausfeld et al., 1998)

and soma position. Namely, KCs with small somata that are born later and arborize in the the anterior calyx where as KCs with large somata (born earlier) arborize in the posterior calyx (Fahrbach, 2006). Similarly this organization is maintained with the small somata forming the core of the bundle, while the ones with the larger somata are in the periphery (Fahrbach, 2006).

It is important to note that even though KCs, might target their dendrites to certain zones in the calyx, it is not necessary that they'll always contact the same postynaptic cells and as result respond to a stimulus in the same way across animals. A recent study in fact has shown that in *drosophila*, KC responses are not sterotyped (Murthy et al., 2008). This in contrast to PNs that reliably target the same glomerulus to contact the same ORNs and produce very similar responses across animals (Jefferis et al., 2001; Masse et al., 2009). Some insights for the differences in the presence or absence of calyx or the number of calyces, come from looking at the input in these species (see below).

INPUT

In most insect species the input to the calyx appears to be strictly olfactory (locusts) [3], while in hymenoptera social insects a large component of the input is visual(Strausfeld et al., 1998; Fahrbach, 2006). In bees there is evidence of gustatory input as well(Campbell and Turner, 2010; Fahrbach, 2006). Lastly MB has been shown to receive neuromodulatory input, that is thought to mediate reward and punishment (Keene and Waddell, 2005).

The importance of MB in olfaction is highlighted in that species that tend to

[3]There is evidence of synaptic contacts formed in the pedunculus. It is therefore important to note that even though KCs might not receive input in the calyces from other modalities, it is possible to receive inputs in the pedunculus and lobes(Fahrbach, 2006)

be anosmic - or have limited need for feeding as adults have smaller MB and lack calyces in most cases (Strausfeld et al., 1998; Fahrbach, 2006) (Figure 1.3) Ultrastructure studies have shown cholinergic and GABAergic input in the MB calyx (Leitch and Laurent, 1996; Fahrbach, 2006). As mentioned in olfactory system section of the introduction, projection neurons in the antennal lobe in insects, receive odor information from olfactory receptor neurons that after processing and formatting they relay to the MB and lateral Horn(LH). In the LH, PNs contact the GABAergic LH internerneurons (LHIs), which in turn send projections back to the MB to inhibit KCs. The role of this feedforward inhibition -as shown in the locust- is to limit the integration window of KCs, contributing to the sparsening of KC responses (Perez-Orive et al., 2002).

Social hymenoptera appear to be an exception to most other insects; in addition to olfactory input, their calyces receive substantial visual input. Medular output from the optic lobe carried through the anterior optic tracy enters enters the MB calyces bilaterally. Olfactory and visual information are segregated in the calyces, targeting different zones within the calyx. In bees olfactory information converges on the lip of the calyx, while visual information is in the collar of the calyx. (Gronenberg, 2001). In addition, there is also a projection from an area of the subeasophageal ganglia that receives sensory afferents from the proboscis and itself targets yet a different area of the MB calyces that does not overlap with visual or olfactory input (Fahrbach, 2006). Therefore the calyces of the bees are characterized as multimodal. Finally, mechanosensory and gustatory input to a small accessory calyx that is separate from the main calyx has been reported for hemimetabolous insects, like the crickets (Fahrbach, 2006)

Studies have suggested that dopamine(DA) and octopamine (OA) are important

modulators that serve as signals for punishment and reward respectively. Dopaminergic and octopaminergic cells have been shown to innervate the MB> Activation of these neurons through genetic manipulation, has been used successfully in place or reward or punishment in order to condition an animal to an odor(Schroll et al., 2006; Claridge-Chang et al., 2009; Keene and Waddell, 2005).

OUTPUT

MB extrinsic neurons connect to the ipsilateral or contralateral MB lobes, where they synapse with KC axons. Such neurons have been identified in the bee, the coachroach, flies and locusts. In most cases the morphology is interesting, but it is not clear what the neuron's function might be. In *drosophila* genetic mutations restricted to some of the extrinsic MB neurons (ex DPM) have been shown to lead to defects in olfactory and memory tasks (Yu et al., 2005; Keene et al., 2006; Krashes et al., 2007). In locusts, recent electrophysiological results provide insights as to how olfactory learning could be accomplished in the presence of a neuromodulator. We examine these results in more detail in the next sections.

1.2.2 MB's role in olfactory learning and memory

Interestingly, the MB appears to be quite plastic. Many studies have shown correlations between behavioral complexity and MB size. In bees, for example, the volume of the MB correlates with the amount of foraging; researchers have shown that worker bees with more foraging experience had larger MB volume when compared with aged matched controls (Withers et al., 1993). This effect is robust having been shown in a variety of bee studies. The idea is that foraging is a visual task that relies on significant formation and accessing of memories (as compared to

staying in the hive) and thus somehow drives the growth of synapses that leads to the enlargement of the MB neuropil. While this increase in MB volume correlates with the behavioral improvement assessed experimentally, no direct causality has ever been demonstrated. Similar experiments have been conducted on other social insects with similar results (Fahrbach, 2006).

The first evidence implicating the MB in a role for olfactory learning came from chemical ablation study. Heisenberg and deBelle's study, used hydroxy urea to kill MB neurons and demonstrate the inability of fly's to learn associations between odors and an electric shock. Specifically, the authors took advantage of the fact that the KC neuroblast is active at a particular time, and by exposing animals to HU during that time the experimenters could almost selectively abolish KCs. The flies were born without MB and while they did not show any abnormalities in locomotion or ability to detect odors, it appeared that they could not associate an odor with an aversive stimulus. The flies appeared unable to use the odor as a predictor for punishment and as such they did not avoid it in a T-maze assay, as normal controls did (de Belle and Heisenberg, 1994).

At the molecular level the cAMP pathway appears to be particularly important for olfactory learning and memory, given that mutants of genes involved in this pathway show deficits in learning/memory olfactory tasks. Some examples include: a)the *dunce* mutant that affects a cAMP-specific phosphodiasterase; b)the *amnesiac* gene that encodes the putative neuropeptide which is similar to pituitary adenylyl cyclase-activating peptide c) mutants affecting protein Kinase A (PKA) and CREB (a transcription factor activated by PKA) in the MB have been shown to affect short and long term memory respectively. d)*rutabaga*. This gene encodes for the calcium/calmodulin dependent adenylyl cyclase. Rutabaga mutants, have memory

deficits in the T-maze assay.

Restoring expression of some of these genes in the MB and/ or some extrinsic MB neurons, has shown to rescue the behavioral deficit observed in these mutants (Davis, 2005) . Interestingly some of these genes have mammalian analogs, that when mutated lead to impairments in spatial memory (Davis, 2005).

These mutant studies have been successful at demonstrating that the mushroom body is involved in olfactory learning and memory. In order to understand,though, how associations between odors and rewards take place, memories are stored etc, one needs to first understand how odors are encoded in the mushroom body and study the neuronal changes that occur during learning. That provides a framework for understanding the effects of manipulations, genetic or otherwise.

1.2.3 Physiology

Consistent with the KCs involvement in olfactory learning and memory, recordings from these cells in locusts and flies have shown KCs to produce sparse responses to odor (Laurent and Naraghi, 1994; Perez-Orive et al., 2002; Stopfer et al., 2003; Turner et al., 2008); they respond to odors rarely, and when they do their response is made of one or two spikes (Laurent and Naraghi, 1994; Perez-Orive et al., 2002; Stopfer et al., 2003; Turner et al., 2008). From a computational point of view, this sparseness facilitates memory storage, recall and modification of memories cite Barlow:1972p3016,Field94,Laurent02,Olshausen04. For a memory to be formed or erased a minimal number of synapses need to be changed. In addition the overlap of odor representations is minimized, such that affecting one synapse holding a memory will have minimal impact on other representations (Marr, 1969; Kanerva, 1988; Laurent, 2002; Olshausen and Field, 2004).

Recently a population of MB extrinsic cells that receive input from KCs in the β lobe (βLNs) (Figure 1.2) has been characterized in the locust using electrophysiological recordings (Cassenaer and Laurent, 2007). It was found that the synapses between KCs and βLNs are governed by spike-time-dependent-plasticity (STDP) (Cassenaer and Laurent, 2007). One of the roles of STDP in this system is the homeostatic control of βLN phase (Cassenaer and Laurent, 2007). Recent results reveled an additional one; when octopamine is injected in the β lobe, it can combine with STDP to selectively change the synapses between KCs and β LNs that STDP has just tagged (Cassenaer and Laurent, in preparation). This is the first experimental demonstration *in vivo* of how STDP could interact with a neuromodulator to selectively affect only those neurons that were responsive to the stimulus (Cassenaer and Laurent, in preparation).

Chapter 2

A Single GABA-ergic Neuron Subserving Normalization for Sparse Encoding in an Olfactory Network

2.1 Introduction

Sparse representations in central brain areas have been observed experimentally in many sensory systems and are likely important for recognition and memory formation (Marr, 1969; Kanerva, 1988; Laurent, 2002; Olshausen and Field, 2004). These representations are carried by a small fraction of the total population of neurons at any given point in time. From a theoretical point of view, sparse representations are advantageous firstly because they increase the storage capacity of the system (Kanerva, 1988; Laurent, 2002). Secondly, sparse representations can extract specific aspects of the stimulus (or in the case of olfaction also form synthetic representations) and make it easier to associate these with higher level areas through learning mechanisms such as Hebbian learning etc (Barlow, 1972; Field, 1994; Laurent, 2002; Olshausen and Field, 2004). Furthermore, manipulation of such representations is highly facilitated in this scheme, since the overlap across representations is minimal, and as such any changes in associations can remain highly specific to the

relevant representation. Finally, such a scheme is energy efficient (Lennie, 2003; Olshausen and Field, 2004).

The importance of sparse representations is highlighted by their presence across different sensory modalities. In the visual system of primates, neurons in area V1 exhibit sparse responses when stimulated with image sequences resembling those that occur during natural vision (Vinje and Gallant, 2000). In the primary auditory cortex of rats, neurons can produce a single spike in response to a sound that is highly reliable across trials (DeWeese et al., 2003). In songbirds, neurons in the nucleus HVC (higher vocal center) respond reliably with one spike at a particular time during the song (Hahnloser et al., 2002). In the olfactory system of insects, Kenyon cells (KCs) respond to odors rarely and briefly (Perez-Orive et al., 2002; Turner et al., 2008). KCs are the intrinsic cells of the mushroom body, an area of the insect brain involved in olfactory learning and memory (Heisenberg et al., 1985; Tully and Quinn, 1985; Davis, 1993). The sparseness of the KC population is attractive from a computational perspective, since these cells are thought to be involved in learning and memory, and their sparse tuning allows for easy handling and storage of memories and limits the overlap across memories (Laurent, 2002). Recently a form of Hebbian learning, spike time dependent plasticity (STDP) was discovered to exist between KCs and their downstream neurons, β-lobe neurons (β-LNs) (Cassenaer and Laurent, 2007).

In this study we examine the role of gain control in maintaining the sparseness of KCs as well as the functional implications for the downstream decoders of KCs, the β-LNs. Gain control is an important computational principle that has been identified at multiple levels of neural processing including coordinate transformations, object recognition, generating invariant responses, and attentional modulation (Andersen

and Zipser, 1988; Salinas and Sejnowski, 2001). It is particularly prominent in sensory systems where the input can vary over several orders of magnitude while the output of neurons encoding the stimuli is limited. Based on previous studies in the lab, I will argue that gain control is necessary at the synapse between PNs and KCs and that a cell we have identified in the locust serves this role. Given gain control's generality across sensory systems, we believe this study to be relevant to sensory coding in general.

The organization of the olfactory system is shallow and it is conserved across phyla (Shepherd, 1994). Briefly, odor detection is carried out by olfactory sensory neurons in the antenna that send axons in the antennal lobe to contact projection neurons (PNs) and local interneurons. PNs transmit information to the next layer; specifically, they send excitatory projections along a tract and contact dendrites of KCs in the mushroom body (MB) and lateral horn interneurons (LHIs) in the lateral horn. LHIs in turn send inhibitory projections to KCs. This feedforward inhibition, together with intrinsic synaptic properties, restricts the KC integration window, making KC act as coincidence detectors (Perez-Orive et al., 2002). Theoretical results (Nowotny, unpublished results) show that in order for KC responses to remain sparse, given the high PN-to-KC connectivity (50%,(Jortner et al., 2007)), there is a clear need for gain control.

Another way to appreciate the need for gain control is to consider how odor concentration is encoded by PNs, the cells presynaptic to KCs. Activity in PNs reflects odor concentration not in global population firing rate- it hardly changes over a 1,000-fold increase in concentration- but in the extent of PN synchronization (Stopfer et al., 2003). Given that KCs act as coincidence detectors, we would expect an increase in odor concentration to lead to a dramatic increase in their firing

rate. In particular, simple calculations show that in the absence of gain control, the relationship between KC population response probability and the number of coincident PN spikes is extremely steep (Jortner et al., 2007). Small deviations of just a few PNs should lead to order of magnitude changes in KC population firing probability, which is not consistent with experimental results (Stopfer et al., 2003). Instead, individual KCs' responses can be selective to a small range of concentrations, or even invariant to concentration, suggesting the existence of gain control.

2.2 Results

We hypothesized that gain control could be provided by a paired giant GABAergic neuron (GGN) that has extensive arborizations throughout the MB (Fig 2.1). This neuron was first described in an electron microscopy study of GABAergic neurons in the locust (Leitch and Laurent, 1996). Intracellular recordings in the locust show GGN to be a non-spiking neuron that responds to all odors tested with graded potential (Figure 2.3). Furthermore, as the concentration of an odor is increased over 6 orders of magnitude, so does the GGN membrane potential, as shown in Figure 2.2i & ii. One way this increase in GGN membrane depolarization can be quantified is by computing the integral of the area under GGN for the duration of the odor response (Fig 2.2vi). Neurotransmitter release in non-spiking cells in the locust has been shown to correlate well with membrane depolarization (Burrows et al., 1982). Taken together these observations suggest that GGN provides increased inhibition as odor concentration is increased (Fig 2.2).

As reported previously, the power of the simultaneously recorded local field potential (LFP) increases with an increase in odor concentration (2.2iii: single LFP

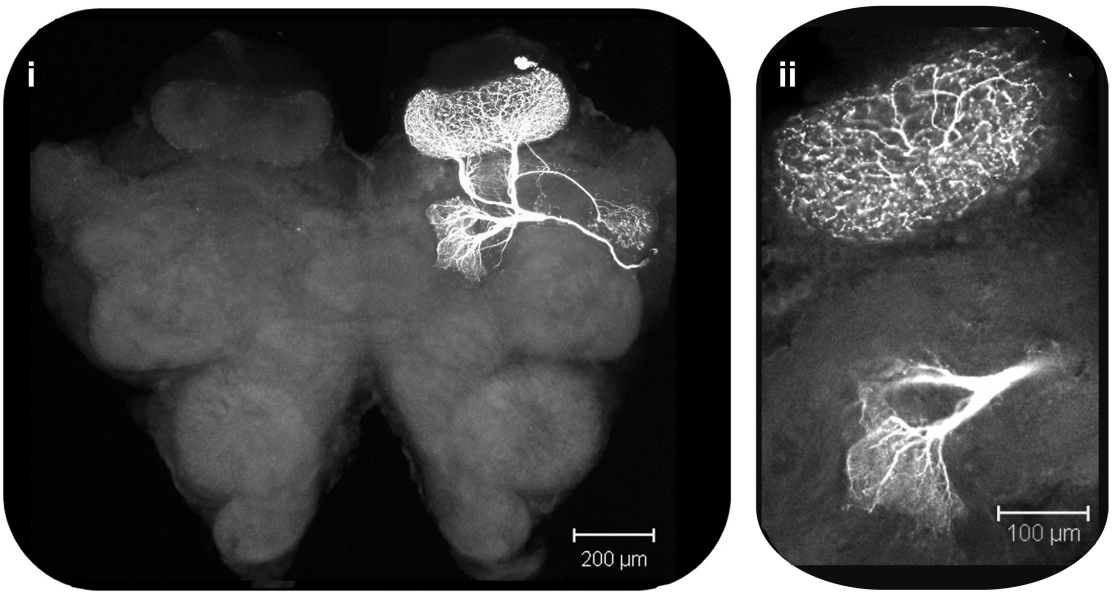


Figure 2.1. GGN Morphology. Intracellular GGN fill with 5% Biocytin. i) An image stack of GGN showing its extensive arborizations in the MB calyx and α lobe, as well as in the lateral horn. Cell body located in the lateral horn. ii)Zoom of an image showing GGN's processes in the calyx (top) and the α lobe (bottom).

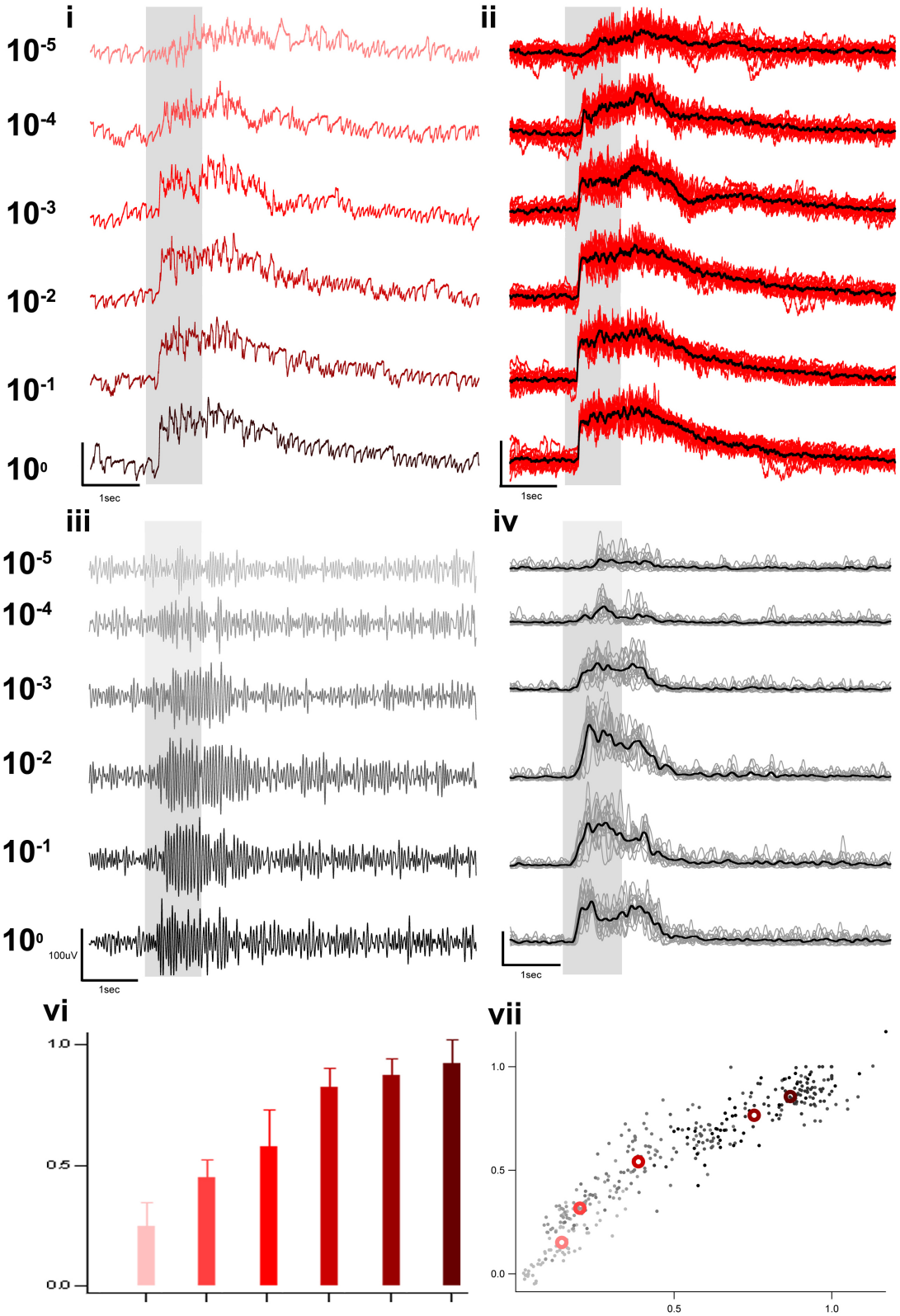
trials, 2.2iv: LFP power, (Stopfer et al., 2003)). The cumulative increase in LFP power during the odor response correlated well with the corresponding increase in GGN activation (measured as the cumulative integral under GGN membrane potential) at the singe-trial level for this experiment and for 5 experiments (n=364 trial pairs over all experiments and concentrations, linear fit, r = 0.93, Fig 2.2vi). Both measures were computed until GGN membrane potential returned to baseline.) Furthermore, the LFP and GGN Vm signals also covary as a function of time: there is a high correlation between the envelope of the LFP for a given trial and the GGN membrane potential for the same trial during odor but not during baseline (Fig 2.3ii, 225 pairs except for air and paraffin oil controls). In other words, we observe that GGN is tracking MB activity with its membrane potential, and we would expect that it provides inhibition over time accordingly.

A closer look at GGN's anatomy (Fig 2.1i&ii), reveals punctate processes in the calyx of the MB, consistent with output, and finer hair-like projections in the α lobe , consistent with input. GGN's anatomy, taken together with the fact that KCs receive input in the calyx and output to the lobes, suggests that GGN could be providing KCs with feedback inhibition.

2.2.1 KC input to GGN

To examine whether KCs indeed provide input to GGN, we recorded intracellularly, simultaneously from GGN and a KC. We evoked spikes in the KC via current injection and recorded the average postsynaptic effect onto GGN. An example of such a spike-triggered average (STA) resulting from 139 event as well as the average from 11 such experiments are shown in Figure 2.4Ai and ii respectively. The data in these experiments (in particular the sharp onset of the EPSP, the delay accounted for by presynaptic spike propagation and the reproducible time-course) demonstrate that KCs are mono-synaptically connected to GGN. Average unitary EPSPs were 0.88 ± 0.50 mV (n = 11 KCs), with some nearing 2mV on average. Extracellular KC stimulation also evokes a depolarization in GGN (Fig 2.4Bi) consistent with

Figure 2.2 ***(on the next page).*** GGN & LFP Different Odor Concentrations Intracellular GGN response and LFP recorded in response to six different concentrations of octanol (listed in left-most column). i) Single trial responses (GGN Vm) for each of the octanol concentrations. ii) 14 Single trials (red) of GGN Vm and corresponding average (black)Biii. Simultaneously recorded LFP (bandpassed 10-30Hz), single trials. iv) LFP power (10-30Hz band) in 14 single trials (grey) and corresponding average (black). vi) Total area under GGN trace (cumulative integral) during the odor response plotted against concentration for experiment shown in B. Mean and standard deviation shown for each concentration. vii) Data from 5 experiments displayed as scatter plot of cumulative LFP power against cumulative area under GGN trace for the duration of odor response for each trial of each concentration. (Dots represent single trials; color coding: light to dark grey represents increase in odor concentration. Circles represent means for each concentration, color coded light to dark red).


i
ii
iii
iv
vi
vii
10-5
10-4
10-3
10-2
10-1
100
1sec
100uV
1.0
0.5
0.0

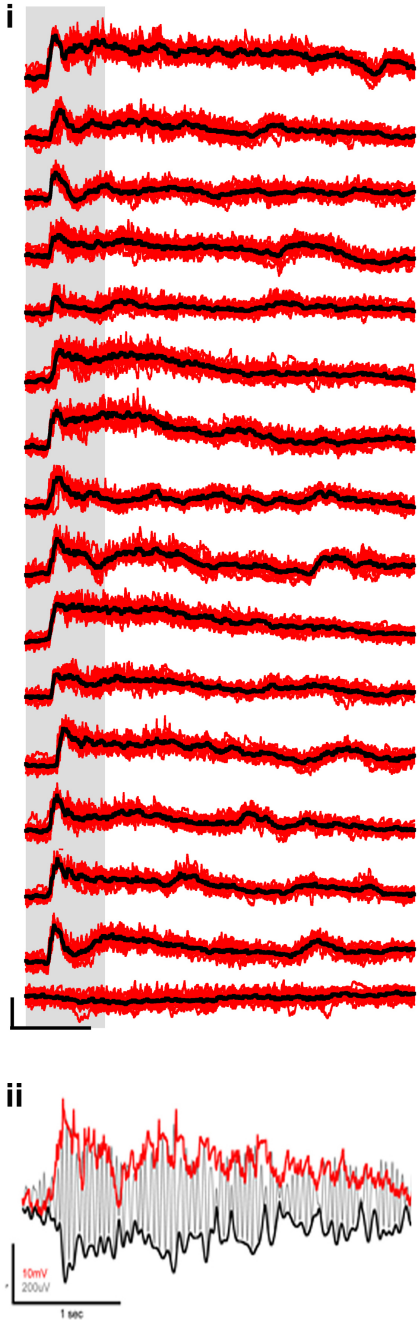
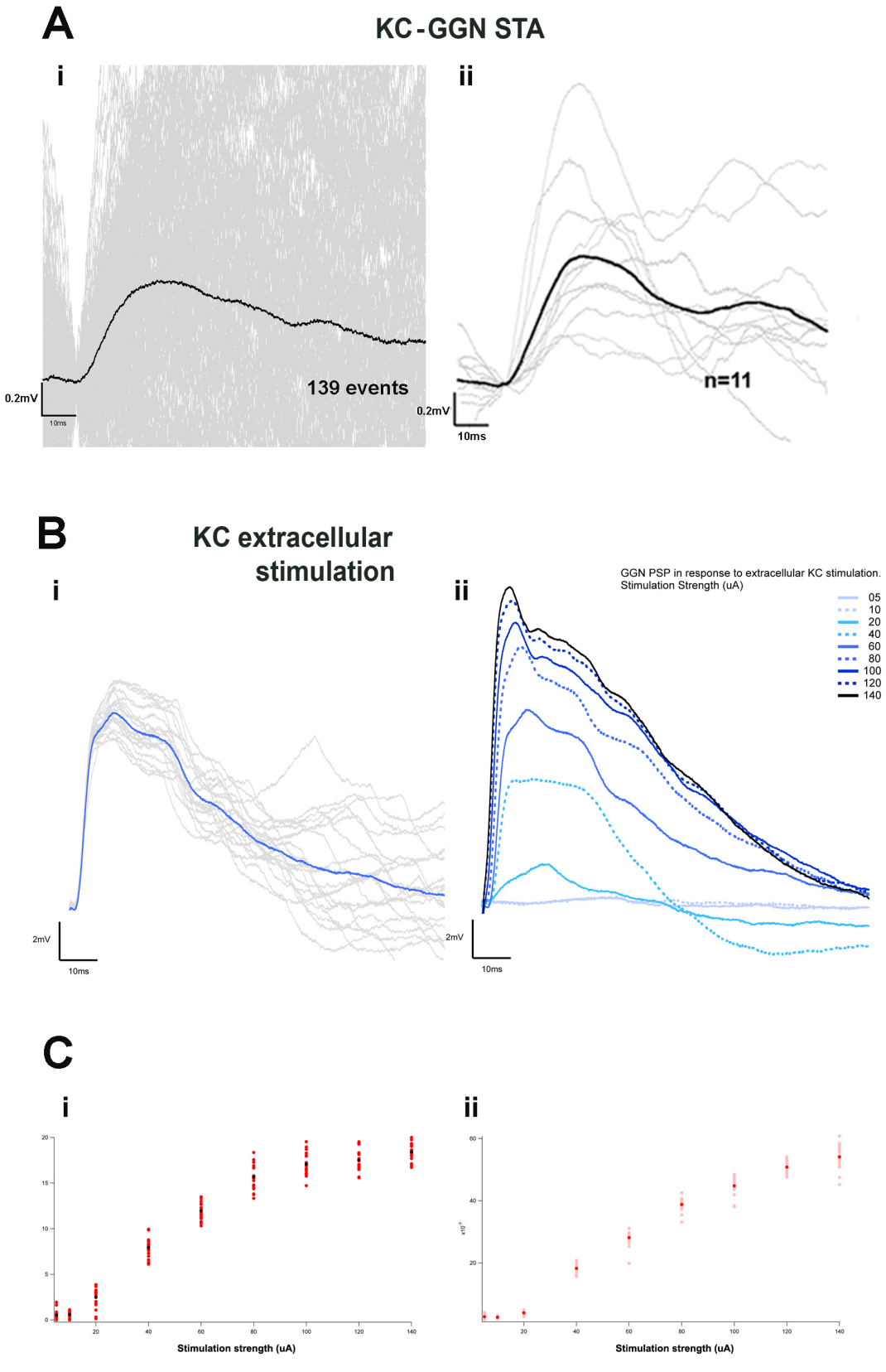


Figure 2.3. GGN Response to Different Odors. i) GGN's response to 15 odors and control. Single Trials (red) of GGN's response to 15 odors and paraffin oil control (last one) and their corresponding average (black). ii) A single GGN trial response (red) from i. with corresponding LFP (grey) and LFP envelope (black).)

the excitatory connection between KCs and GGN. In addition, KC extracellular stimulation allowed us to assess the effect on GGN of activating multiple KCs simultaneously. Increasing stimulation strength (5-140uA), thereby presumably activating more KCs, led to an increase in PSP amplitude on GGN with a peak between 15 and 20 mV (Fig 2.4Bii). This effect was observed whether we measured the peak amplitude of the PSP (Fig 2.4Ci), the maximum slope (Fig 2.4Cii) or the area under the curve. This effect was measured across 6 experiments for 8 different stimulation channels. As such, it appears that activating more KCs increases the extent of depolarization in GGN.

Lastly, we asked whether input from KCs alone is sufficient to account for the sustained depolarization observed in GGN's odor response. As described in the introduction, olfactory stimulation gives rise to oscillatory population activity in the antennal lobe, which propagates to the MB, evoking sparse volleys of approximately simultaneous KC spikes. Such a profile of KC activity can be readily mimicked with extracellular stimulating electrodes, allowing us to address the question we posed. We observe that stimulation of KCs at a frequency similar to the odor-evoked oscillations reproduces the observed DC shift, suggesting that excitatory input from KCs onto GGN can account for GGN's excitatory response during odor (Fig 2.5).

Figure 2.4 ***(on the next page).*** Excitatory Input to GGN. **A.** KC-GGN STA. Ai: example of a KC-GGN STA (black, average of 139 raw Vm events in grey). Aii: Average (black) of 11 such average STAs, each from a different KC (grey). **B.** GGN PSP in response to extracellular KC stimulation Bi: Raw (grey) and average(blue) PSP in GGN following KC stimulation (60uA). Bii: Increasing stimulation strength over a large range of stimulation strengths (5-140uA, including 60uA from Bi) results in increased PSP size recorded in GGN (each trace is an average of 20 trials); **C.** Quantifying the effect of increasing KC stimulation strength on GGN PSP for experiment in Bii. Ci: Peak amplitude of GGN PSP plotted versus stimulation strength; Cii: GGN PSP slope plotted versus stimulation strength.


A
KC-GGN STA
i
ii
139 events
n=11
0.2mV
10ms
0.2mV
10ms
B
KC extracellular stimulation
i
ii
GGN PSP in response to extracellular KC stimulation.
Stimulation Strength (uA)
05
10
20
40
60
80
100
120
140
2mV
10ms
2mV
10ms
C
i
ii
Stimulation strength (uA)
Stimulation strength (uA)

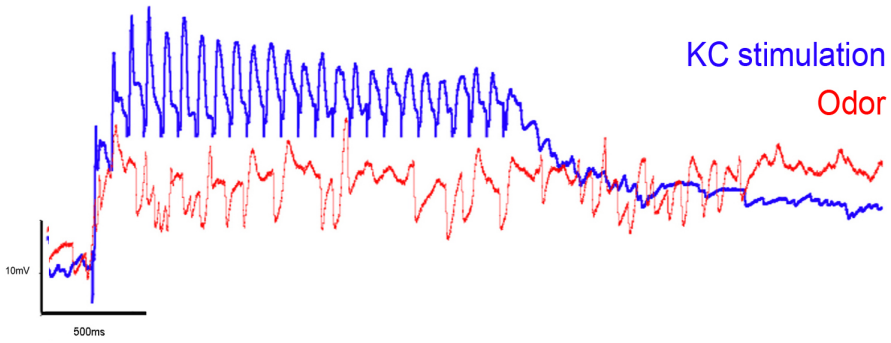


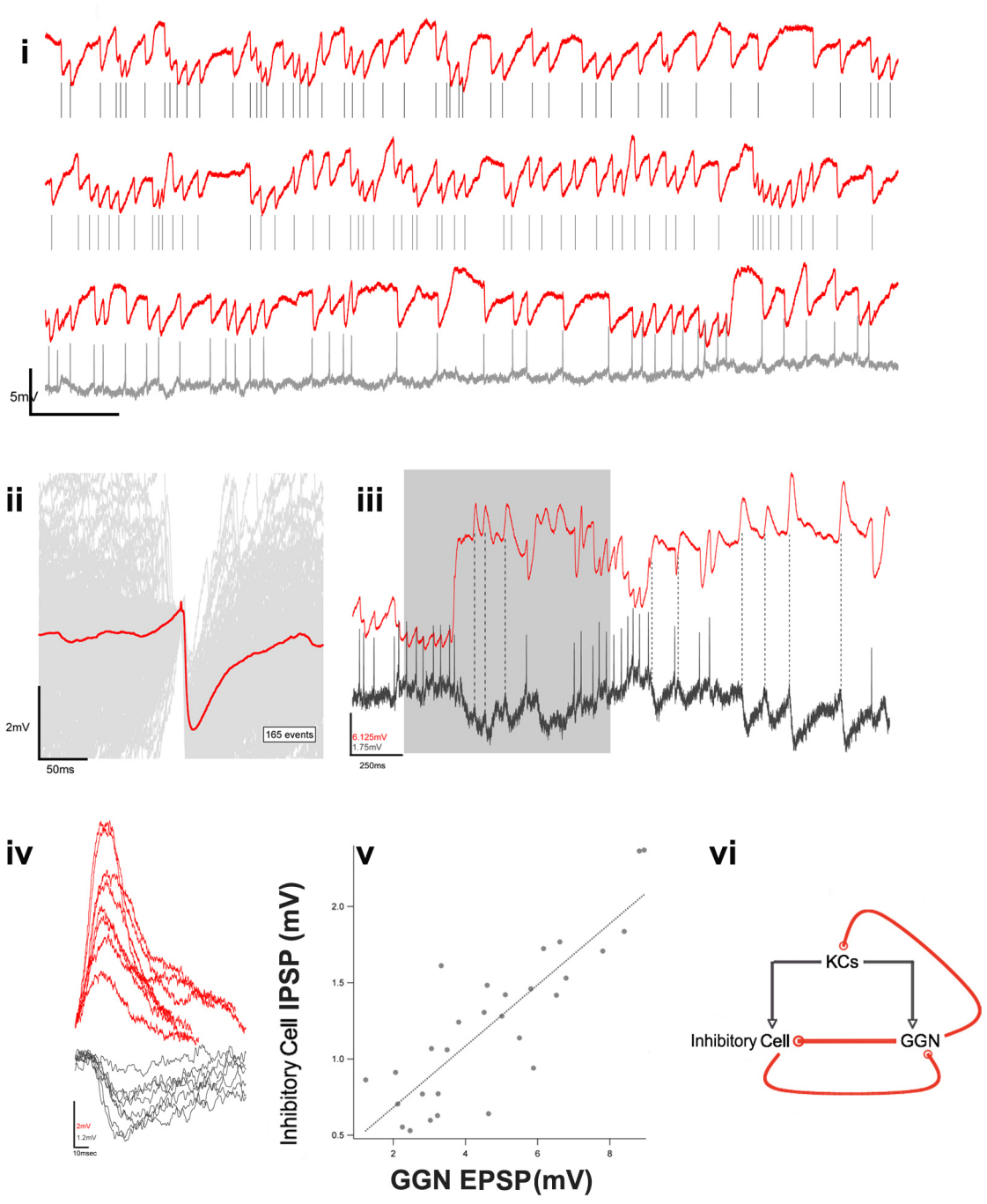
Figure 2.5. DC Shift. GGN response to a train of KC stimulation at a frequency similar to the dominant frequency recorded in the LFP during odor stimulation (25 pulses at 25Hz), overlaid with an example of the odor response for the same recording.

The PSP onto GGN evoked by KC stimulation above a certain threshold is not a pure EPSP; rather it appears that the excitatory effect is followed by a delayed inhibitory effect, suggesting that KCs might provide input to another cell, which provides delayed inhibition to GGN. Inhibition onto GGN is very prominent and particularly noticeable during baseline as a constant barrage of IPSPs (Fig 2.6i). If GGN releases in a graded manner, the role of this inhibition onto GGN could be to limit GABA release from GGN in the absence of odor stimulation. We identified the source of this inhibition to be a neuron with processes in the β-lobe. Given that KC axons terminate in this area, perhaps the simplest explanation for the inhibition observed in GGN upon KC stimulation, is that this neuron receives direct input from KCs. The action potentials generated by this neuron can account for all IPSPs observed in GGN during baseline and odor (Fig 2.6i & iii). An STA generated from the spikes of this cell during baseline is shown in Fig 2.6ii. A closer look at the odor response of the simultaneously recorded cells reveals that GGN not only receives inhibition from this neuron, but that, when depolarized, GGN provides inhibition back onto this cell (Fig 2.6iii &iv). Consistent with GGN releasing in a graded

fashion, the size of the IPSP on this inhibitory neuron increases with increased GGN depolarization (Fig 2.6v).

Thus far, we have identified KCs as the source of the excitatory input to GGN and the inhibitory cell as providing the prominent inhibition to GGN. Furthermore, GGN forms a feedback loop with this inhibitory cell (Fig 2.6vi), thus providing an additional layer of control. Next, we sought to investigate whether GGN outputs onto KCs so as to complete the feedback loop.

Figure 2.6 ***(on the next page).*** Source of inhibitory input to GGN. i) Intracellularly recorded traces of GGN (red) and inhibitory cell (grey) during baseline. Each IPSP in GGN is accounted for by an action potential in the inhibitory cell. ii) Corresponding STA for i. iii) Intracellularly recorded traces of GGN (red) and inhibitory cell (grey) during odor. GGN provides inhibition back onto this cell; grey bar: odor ON. iv). Examples of GGN PSPs with corresponding IPSPs on inhibitory cell. v) Scatter plot showing the correlation between the size of the GGN PSP and the corresponding IPSP on the inhibitory cell. vi) Schematic showing relationship between GGN and its inputs)


i
5mV
ii
2mV
50ms
165 events
iii
6.125mV
1.75mV
250ms
iv
2mV
1.2mV
10msec
v
Inhibitory Cell IPSP (mV)
GGN EPSP(mV)
2.0
1.5
1.0
0.5
2
4
6
8
vi
KCs
Inhibitory Cell
GGN

2.2.2 GGN output onto KCs

Since GGN is GABAergic, we wanted to verify that its effect is inhibitory; and if so, to determine whether it exerts its effect presynaptically onto PN terminals and/or postsynaptically onto KC dendrites. If it has a direct postsynaptic effect on KCs, we should be able to affect spiking in KCs even in the absence of PN excitation. To test this we performed dual intracellular recordings; we used current injection of 70-300 pA to evoke 3-4 spikes in a KC and sought to eliminate them in alternating trials by depolarizing current injection into GGN (schematic Fig 2.7B). Since the electrotonic nature of this nonspiking neuron is unknown, and the effect of current injection at one location on distant release sites is impossible to predict without empirical data, we first performed simultaneous intracellular recordings at two distinct locations along the GGN dendrite (Fig 2.7A). The highest current injected (19.5nA) in the first GGN location appeared to evoke a depolarization in the second location that was comparable in amplitude to the odor response in the second location (Fig 2.7A). Furthermore, intermediate current values (1.5, 5.5, 13.5, 15.5, 17.5nA) produced intermediate depolarizations[1] in GGN (Fig 2.7A). We tested the effect of GGN depolarization on the suprathreshold activation of individual KCs by direct current injection. A KC was impaled in its soma, and a current pulse (150pA, 200 ms) was injected to evoke ~5 action potentials on average. Figure 2.7B, shows these trials with one KC. Following each pulse, we combined the depolarizing pulse into the KC with the depolarization of GGN, by

[1] In the ideal case, we would have been able to measure the voltage deviation at the current injection site as well, and thus have two voltage deviation measurements. With these, we would have been able to measure the transfer resistance between the two recording sites. In this case, it was not possible to do so, because the input resistance of the cell is low, and the currents injected very large. As a result of the high electrode resistance, there is a strong nonlinear rectification at the electrode, and the bridge cannot be entirely balanced. Hence transfer resistance could not be measured.

current injection (3.5, 11.5 or 19.5nA, same pulse duration as KC) through a second microelectrode placed in a dendrite of GGN. An injection of 19.5 nA was known to evoke a depolarization of 10mV at a distant site, commensurate with an odor evoked GGN response (see above). The average effect for injecting 19.5 nA was a ~55% reduction (n=8 KCs). As shown in Figure 2.8i, the extent of the effect on KC firing is dependent on the amount of current injected into GGN.

Having shown that KC stimulation can depolarize GGN, and that injecting current in GGN can eliminate current-evoked KC spikes, we next tested a variant of this experiment. In this case, the depolarization of GGN was caused not directly by current injection, but by synaptic drive from KCs. Indeed, if GGN adaptively regulates KC population output, the extent of its own depolarization should depend on KC activity. We thus kept one intracellular electrode in one KC, to be used as our assay for the effect of GGN depolarization. We kept a second electrode in a GGN dendrite to assess its membrane polarization. Finally, we added a pair of extracellular electrodes in the KC soma region, far from the KC impaled for intracellular recording, to stimulate a new set of KCs and thereby indirectly depolarize GGN (Fig 2.7C). As expected based on the previous results, we saw a reduction of KC firing also with this manipulation (n= 8, average effect ~85% reduction, Fig 2.7C). We also found that the decrease in intracellularly recorded KC firing rate was dependent on the strength of extracellular stimulation of the other KCs (Fig 2.8ii). Simultaneous intracellular recordings of GGN, showed that the extent of its depolarization correlates well with KC stimulation strength and also with the extent of the decrease in KC firing rate (Fig 2.8ii). This manipulation was more effective in eliminating KC spikes compared to direct current injection into GGN (~85% vs ~55% reduction, ratio of slopes Fig 2.8ii/Fig 2.8i =1.76), arguably because synaptic

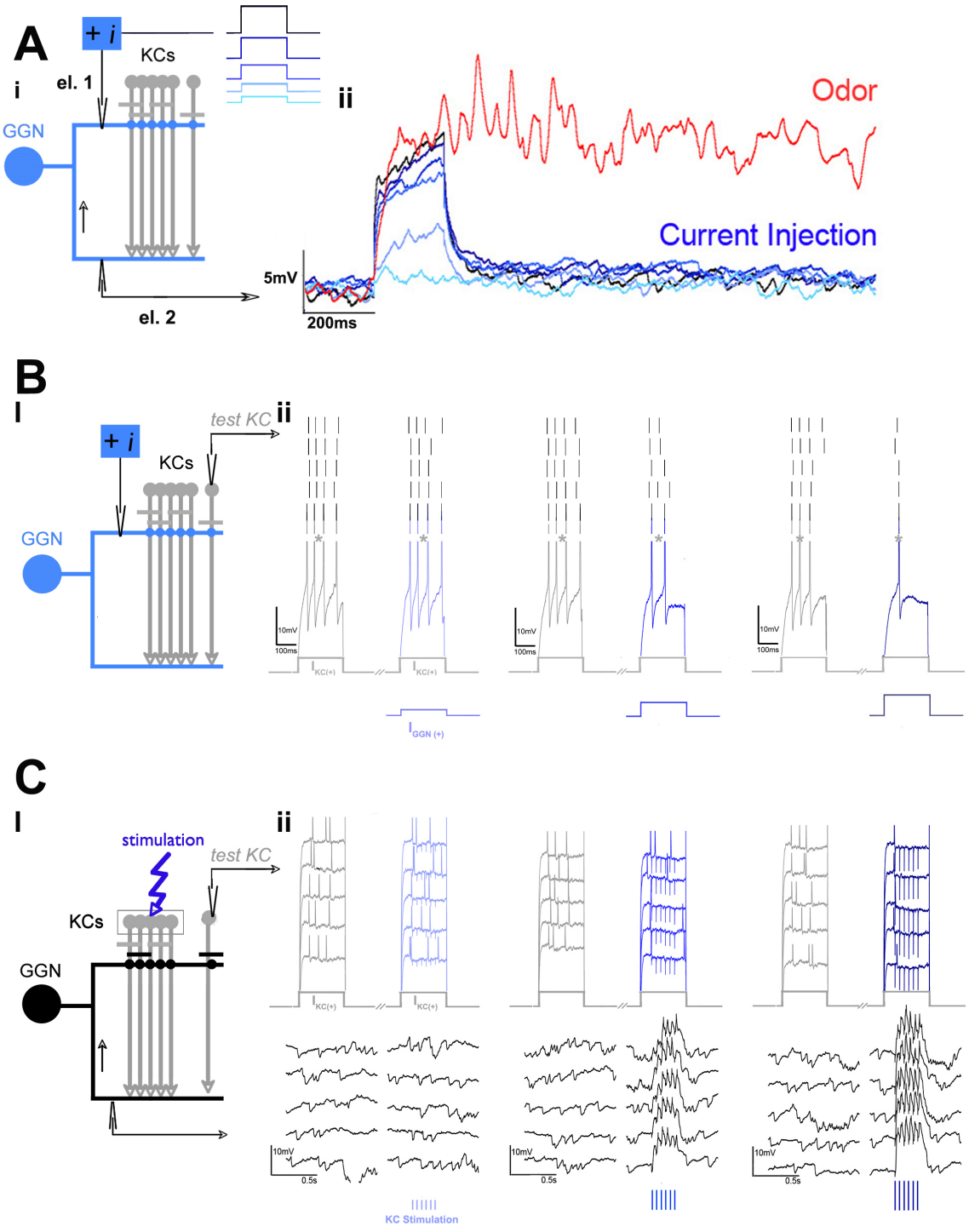
input to GGN might evoke release more readily than artificial current injection at an arbitrary location within the neuron.

To summarize, we have demonstrated that the degree to which KC output is reduced by GGN activation is related, in a positive way, to the degree to which KCs are excited. Also, we have shown that GGN's actions are consistent with a normalization or adaptive control of the KC population output. The gain of this loop, however, is unknown and how compressed the KC output range is remains unknown.

2.2.3 Manipulation of GGN state during odor stimulation

To gain insight into its function, we injected current into GGN during odor presentation while monitoring input and/or output from KCs (we use input to refer to subthreshold effects and output to mean KC action potentials). By imposing an artificial de- or hyper-polarization of GGN by direct current injection, we perturbed the KC-GGN feedback loop and assessed the effect on KC activity.

Figure 2.7 ***(on the next page).*** GGN output onto KCs. **A.** Intracellular recording of GGN in two different locations. Ai) Schematic summarizing the experiment. We injected current pulses of different amplitude in one GGN location and recorded the effect in a second GGN location (Vm at second location shown in blue in Aii). Odor response at second location (red) overlaid for comparison. **B.** Injection of depolarizing current into GGN can eliminate current-induced KC spikes Bi) Diagram explaining the manipulation; Dual intracellular recordings of GGN and a KC. (Bii) Two current pulses (per trial) in an intracellularly recorded KC evoke spikes (raster and Vm of last trial shown); one pulse is paired with current injection in GGN. Current Injected in GGN from left to right: 3.5nA,11.5nA & 19.5nA. Star: spikes were clipped by 20mV. **C.** KC stimulation can eliminate current-induced KC spikes (through indirect depolarization of GGN). Ci) Diagram explaining the manipulation; Dual intracellular recordings of GGN and a KC recording the effect of exciting a different set of KCs with extracellular stimulation. Cii) Two current pulses (per trial) in an intracellularly recorded KC evoke spikes; one pulse is paired with a train of stimulation of a different set of KCs (KC spikes are clipped.) Stimulation strengths (from left to right): 10uA, 20uA & 30uA. Note: Downward deflections in KC traces are stimulation artifacts.


A
i
+ i
KCs
el. 1
GGN
el. 2
ii
Odor
Current Injection
5mV
200ms
B
I
+ i
KCs
test KC
GGN
ii
10mV
100ms
I KC(+)
I GGN (+)
C
I
stimulation
test KC
KCs
GGN
ii
10mV
0.5s
KC Stimulation

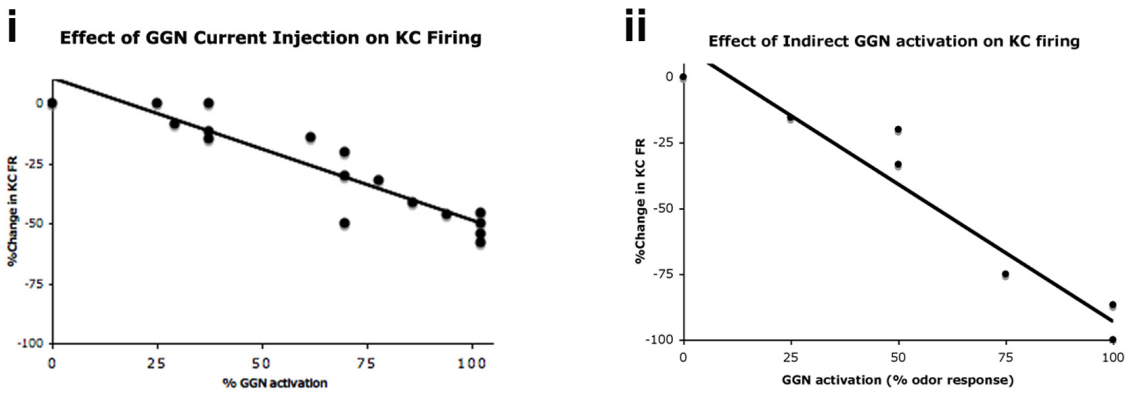


Figure 2.8. Summary data for multiple experiments. i) Scatter plot of 5 KCs & a linear fit of percent reduction in KC firing as function of increasing GGN activation. The KC reduction is computed with respect to the control KC pulse included in the same trial. To compute % GGN activation, the current injection values are rescaled based on their effect on GGN Vm, as assessed in dual GGN recordings, and normalized to the highest value (see suppl Fig3). ii) Scatter plot of 3 KCs & a linear fit of percent reduction in KC firing as a function of increasing KC stimulation and thereby GGN activation.The KC reduction is computed with respect to the control KC pulse included in the same trial. Stimulation strength is translated to 0, 25, 50, 75 or 100% of a high odor response in the same location.

Injecting depolarizing current into GGN during the odor strongly affected subthreshold KC activity. In experiments where positive current was injected in GGN during odor stimulation (alternating trials with and without GGN current injection) LFP power in the 10-30Hz band was reduced to 15% of the odor control (Fig 2.9A). The opposite effect, i.e an increase in LFP power (10-30Hz band, 125% of control) was observed when GGN activity was reduced by negative current injection (Fig 2.9Aiii&iv). Although the origin of the LFP oscillations in the 10-30Hz band is in the AL, and arises from interactions between PNs and LNs, the actual signal measured in the MB is thought to represent synaptic currents flowing into KCs. As shown in Figure 2.7, GGN, in addition to PNs, also provides input to the KCs. It appears, therefore, that by injecting depolarizing current into GGN we reduce the effectiveness of PN input into KCs. Intracellular recordings of single KCs (Fig2.9B) confirm the results we observe with the global LFP measure. Specifically, the power

in the 10-30Hz band in the KC membrane potential is also reduced to about 15% of control when depolarizing current was injected into GGN. An example of such a recorded KC is shown in Figure 2.9B. Conversely, reducing GGN activity during odor leads to an increase in the power of the KC membrane potential by 130% (Fig 2.9Biii & Biv). The effect observed in both LFP and subthreshold KC activity could be mediated not only by altering KC membrane potential, but also by changing KC input conductance and thus, time constant and reactiveness to synaptic driveÑmore generally, KC integrative properties. Such action would be nonlinear, and be akin to a shunt on PN drive onto KCs. To ensure that our results derived from current injections into GGN do not have an artifactual component, we carried out control measurements following the GGN recordings, where current of either polarity was injected 50-100 microns outside GGN. These manipulations do not give rise to the changes we see when current is injected into GGN (Fig 2.10B).

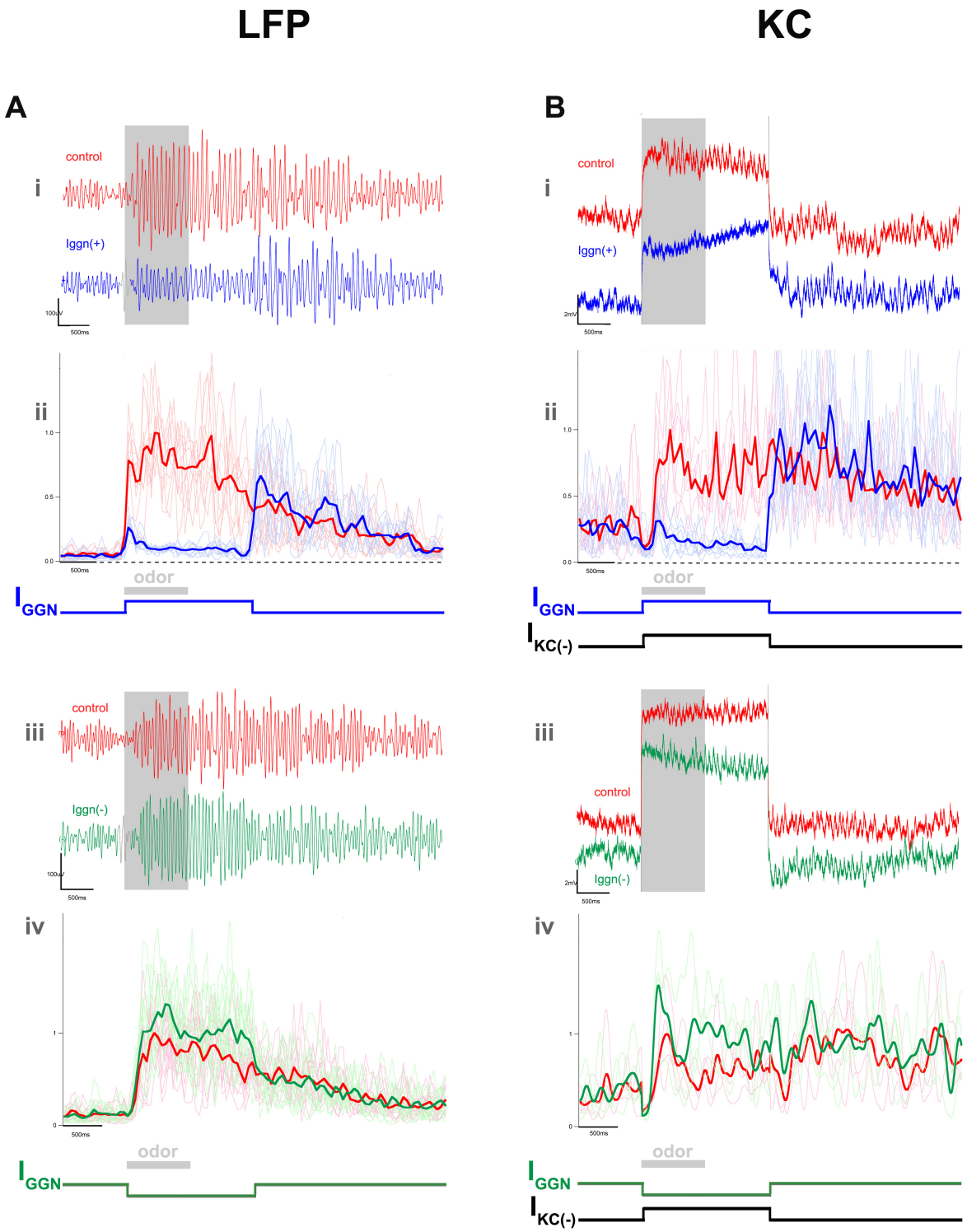
How is KC output affected?

Given these changes in KC subthreshold activity, we wondered to what extent KC spiking would be affected. Because only a small fraction of KCs respond to an odor with action potentials, we have relatively few recordings where we can assess the effect of manipulating GGN activity during odor-evoked KC firing. Moreover, the total KC population is quite large (50,000/MB), and it would be desirable to sample a reasonably large fraction of the KC population to conclusively assess the effect of GGN on KC output.

To resolve this issue, and to evaluate GGN's functional role in the circuit, we recorded intracellularly from β-lobe neurons, which take direct input from KCs. This is a small population of neurons ($\sim$ 30), each receiving input from a large number of KCs ($\sim$ 5,000). Assessing the extent of GGN's influence on this downstream

population of neurons could further reveal the relative importance of GGN in this circuit. β-LNs have a low baseline firing rate, but respond quite vigorously during the odor (Cassenaer and Laurent, 2007). We find that manipulating GGN activity during odor greatly impacts β-lobe neuron (β-LNs) firing. In particular, injection of depolarizing current into GGN could led to a complete shutdown of the β-LN's firing during odor in 6 out of 8 βLNs recorded (Fig 2.10A & B, and a 96% and 82% reduction in 2/8). GGN exerts its effect on β-lobe neurons indirectly by acting on KCs, since positive current injection in GGN is incapable of eliminating any current induced β-lobe neuron spikes in the absence of odor (n=3 cells). Conversely, injecting hyperpolarizing current into GGN during odor in the same cell had the opposite effect, namely increase its firing rate to 140% of control.

Figure 2.9 ***(on the next page).*** Effect of manipulating GGN activity on KC activity during odor stimulation: **A.** Effect of manipulating GGN activity during odor on LFP power: Ai & Aii) Enhancing GGN activity by positive current injection (Iggn), leads to a decrease in the LFP power. Ai) 2 example traces (bandpassed 10-30Hz) recorded consecutively in response to odor and positive Iggn (blue) or odor alone (red). Aii) Power in the 10-30Hz band for each of the two different conditions (20 interleaved trials, light blue & pink) and their respective average (red & blue). Aiii & Aiv) Reducing GGN activity by negative current injection (Iggn) leads to an increase in LFP power. Ai) 2 Example traces (bandpassed 10-30Hz) recorded consecutively in response to odor and negative Iggn (green) or odor alone (red). Aii) Power in the 10-30Hz band for each of the two different conditions (20 interleaved trials, light green & pink) and their respective average (red & green). **B.** Effect of manipulating GGN activity during odor on KC subthreshold membrane potential oscillations: Bi & Bii) Enhancing GGN activity by positive current injection (Iggn), leads to a decrease in the power of KC subthreshold membrane oscillations. Bi) 2 Example traces recorded consecutively in response to odor and positive Iggn (blue) or odor alone (red). Bii) Corresponding power in the 10-30Hz band for each of the two different conditions (20 interleaved trials, light blue & pink) and their respective average (red & blue). Biii & Biv) Reducing GGN activity by negative current injection (Iggn) leads to an Increase in the power of KC subthreshold membrane oscillations Bi) 2 Example traces recorded consecutively in response to odor and negative Iggn (green) or odor alone (red). Bii) Corresponding power in the 10-30Hz band for each of the two different conditions (20 interleaved trials, light green & pink) and their respective average (red & green). KCs are held hyperpolarized with a very small amount of hyperpolarizing current during the trial except for the duration of Iggn, when there is no current injected in the KC.

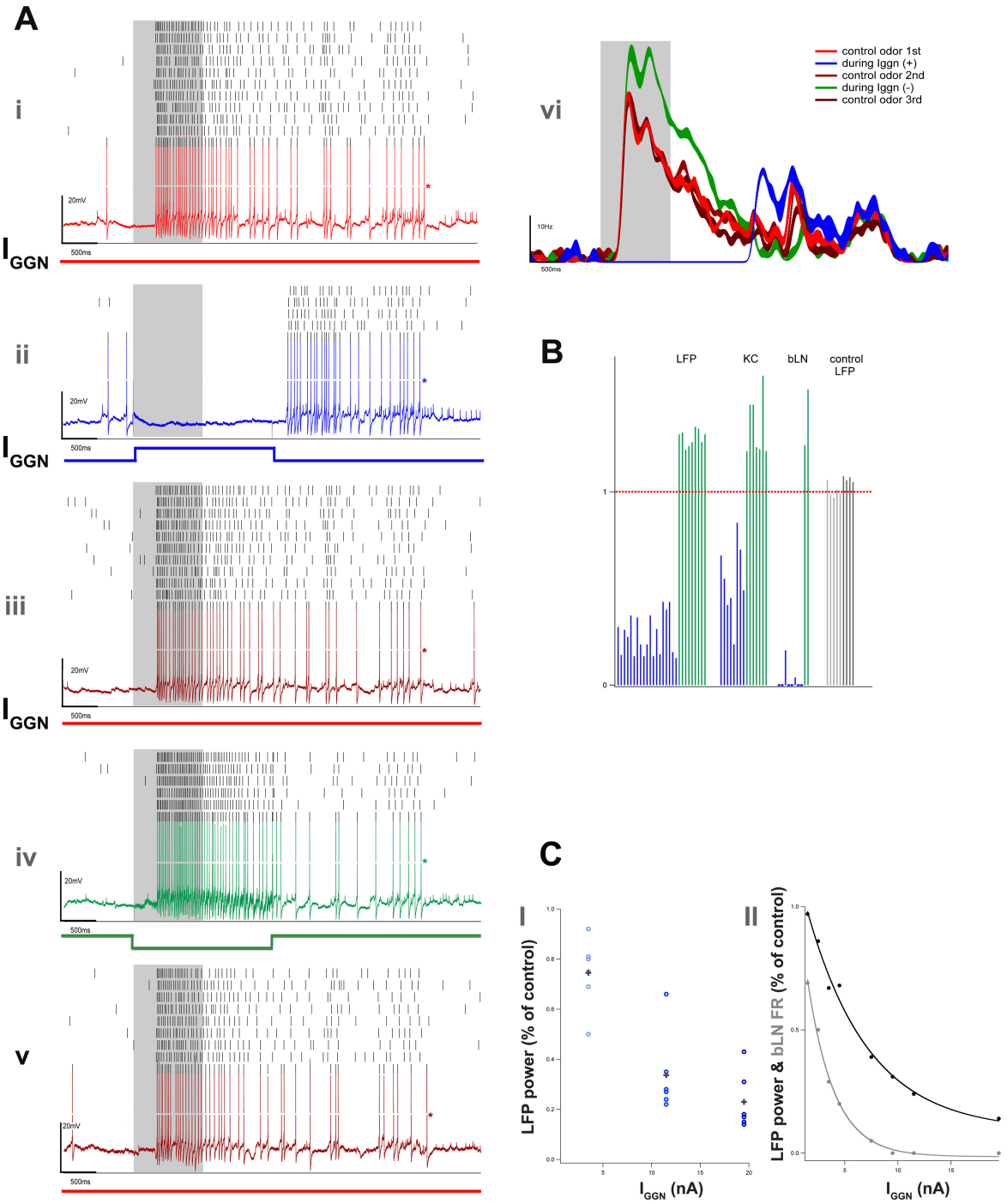

LFP
KC
A
B
i
control
Iggn(+)
ii
odor
IGGN
IKC(-)
iii
Iggn(-)
iv

The effect of all GGN manipulation experiments during odor on LFP and KC power, and on β-LN firing are summarized in Figure 2.10B. The % values shown are with respect to averaged preceding & following control trials.

Lastly, I quantified the effect on LFP power and bLN firing as a function of the amount of current injected into GGN (Fig 2.10C). Given GGN's graded odor response we reasoned that injecting depolarizing current into GGN at intermediate levels during the odor should result in an intermediate effect on KC activity. Testing this prediction with 3 current injection values in GGN we observe corresponding changes in LFP power, confirming this hypothesis (Fig 2.10Ci, n=5 experiments). We also observed this graded effect in an experiment where we quantified the effect of a wider range of current injected into GGN on both LFP power and β-LN firing. These results are consistent with GGN's effect on current-induced KC firing in the absence of odor stimulation (Fig 2.7 B & C).

Figure 2.10 ***(on the next page).*** Effect of manipulating GGN activity on KC activity during odor stimulation:**A.** Effect of manipulating GGN activity during odor on β-lobe neuron firing Ai-Av) Raster plots of the same β-LN (Vm of last trial also shown, spikes are clipped.) in response to odor alone (Ai, Aiii, Av) or odor and positive Iggn (Aii) or negative Iggn (Aiv). Trials are shown in the order in which they were recorded. Avi) Corresponding smoothed PSTHs +/- SE (β-LNs spike times convolved with a 50ms Gaussian). **B.** Summary data for multiple experiments: effect of positive (blue) or negative (green) Iggn during odor for LFP, KCs and β-lobe neurons. Experimental control: Effect on LFP for positive (light grey) or negative (dark grey) current injection 50-100uM outside GGN.In all cases, values shown are expressed as% of responses recorded to odor alone. **C.** Effect of increasing amplitude of positive Iggn on LFP power & β-LN firing. Ei) Summary data for multiple experiments: 3 different amplitudes of positive Iggn on LFP power (n=5 exp) plotted here as % of interleaved control odor trials. Eii) One experiment examining the effect of multiple current injection values in GGN and their effect on simultaneously recorded LFP power and β-LN firing rate. Both are expressed as % of control trials (average of those odor trials directly proceeding and following the manipulation trials). For the β-LN, spike times were converted to smoothed PSTH as in Avi and total change over the duration of Iggn was compared. Notes: grey bar: odor ON (1sec)

β-LN


A
i
ii
iii
iv
v
20mV
500ms
I GGN
vi
control odor 1st
during Iggn (+)
control odor 2nd
during Iggn (-)
control odor 3rd
10Hz
500ms
B
LFP
KC
bLN
control LFP
C
I
LFP power (% of control)
I GGN (nA)
II
LFP power & bLN FR (% of control)
I GGN (nA)

2.3 Discussion

In this study I describe how gain control is employed to maintain sparseness of a population of neurons involved in olfactory memory. The source of this gain control is a single neuron (per hemisphere) implementing a feedback loop to provide each KC with inhibition that is proportional to the total activity of the KC population. I show that as I activate more KCs, GGN becomes more depolarized and in turn provides more inhibition back onto KCs. I also describe an additional feedback loop between GGN and another inhibitory cell that itself also receives input from KCs. This extra feedback loop highlights the level of control in the system and the importance of regulating the network not only during odor, but also during baseline. The high firing rate of this neuron during baseline suggests that is important to limit neurotransmitter release by GGN in the absence of odor stimulation.

To gain insight into how GGN controls the gain of the KCs during sensory stimulation, I perturbed the feedback loop that exists between KCs and GGN by manipulating the extent of GGN activation beyond the level evoked by the stimulus alone. Injecting depolarizing current into GGN during odor stimulation dramatically reduced the subthreshold oscillations observed in KCs, as assessed both at the single-cell level and with the global LFP measurement. Conversely, injecting hyperpolarizing current into GGN had the opposite effect The effect of GGN is more considerable still when measured on the output of the MB. By injection of depolarizing current into GGN we can effectively and reversibly shut down essentially the entire output of the MB. Given the KCs' involvement in learning and memory, we hypothesize that the graded manipulation of GGN would measurably impair learning or memory recall at the behavioral level.

GGN acts to control the gain of KCs: as the concentration of an odor increases,

so does the synchrony among PNs, which would dramatically increase KC firing if it were not kept in check.

By increasing KC input resistance, through shunting of PN excitatory input, GGN contributes to keeping KC responses within a certain dynamic range that is suitable for downstream neurons and in this way provides gain control. Shunting inhibition has been proposed as a mechanism underlying divisive scaling of neuronal activity that can serve as an implementation of gain control (Carandini and Heeger, 1994). Several studies have examined the conditions under which this could happen. Modeling and electrophysiological studies have shown that when the excitatory input is tonic, in the absence of noise, shunting inhibition has a subtractive rather than divisive effect on neuronal activity (Holt and Koch, 1997). Under conditions where there is high variability in the synaptic input (Mitchell and Silver, 2003; Prescott and Koninck, 2003) or there is synaptic noise arising from strong balanced excitation and inhibition (Chance et al., 2002), shunting inhibition provides divisive scaling. If the inhibition is implemented as feedback, it results in divisive scaling of neuronal activity, regardless of the noise in the system (Sutherland et al., 2009).

We cannot exclude that GGN also exerts its effect via presynaptic inhibition [2] of PNs in the calyx, as we don't have at present a method for evaluating that possibility. Given the results shown in Figure 2.7, i.e. GGN's ability to eliminate current-evoked KC spikes in the absence of odor stimulation, we believe that shunting inhibition accounts for at least part of GGN's effect on KCs.

Why is the activity of the MB set within this particular range?

We have already addressed this question from the point of view of neural coding, and in terms of the synaptic changes required to store a memory. An additional

[2] It was shown recently that local interneurons control the gain of PNs in the AL by presynaptic inhibition of ORN input to PNs (Olsen and Wilson, 2008)

perspective on this issue comes from the neurons that decode KC activity patterns, and in particular, what is known about the rules governing synaptic connections between them. We hypothesize that GGN activity is set at its current level, such that it ensures that the KC output causes β-lobe neurons (β-LNs) to fire (mostly) only one AP per LFP oscillation cycle. The synapses made by KCs onto this class of extrinsic MB neurons are governed by STDP, which synchronizes the β-LN population by maintaining their firing at a particular phase of the LFP oscillation cycle during the odor response. Specifically, if a β-LN fires earlier than its typical phase, STDP will act to depress the KC synapses that contributed to the AP, so the next time that the same inputs are activated the cell with fire later. Conversely if the βLN action potential arrives later than the preferred phase, its inputs will become mostly potentiated, which will serve to advance the β-LN AP. The effect of STDP is of particular interest in light of the effect on β-LNs of reducing GGN activity (via current injection). During this manipulation we observed that the firing rate of β-LNs increases, and β-LNs fire multiple APs within a given LFP oscillation cycle. Due to this erratic firing, the β-LN is no longer able to recover the appropriate firing phase or rate, and as a result, the population would effectively become de-synchronized. We therefore hypothesize that GGN maintains the KC output within a range that allows STDP to carry out its homeostatic fine-tuning.

2.4 Methods

2.4.1 Preparation and stimuli

All results in this study were obtained *in vivo* from locusts (*Schistocerca americana*) that are housed in an established, crowded colony. Young adults of either sex were

immobilized in a holder. Both antennae were secured in place with respect to the olfactory delivery system and remained intact for olfactory stimulation. The brain was exposed, desheathed and superfused with locust saline, as previously described (Laurent and Naraghi, 1994). The results presented here originate from 80 recordings of 55 GGN, 30KCs and in 10 β-LNs in 55 locusts.

2.4.2 Odor Delivery

Odors were diluted 10% volume/volume in paraffin oil. Different odor concentration were prepared by serial dilutions, ie 10^{-2} is 1:100 dilution of odor in paraffin oil. Odors were delivered by injection of a controlled volume of odourized air within a constant stream of dessicated air. Total airflow was set t 0.85 L/min and the odor was further diluted by 1/3 in air. Teflon tubing was used at and downstream from the mixing point to prevent odour lingering and cross-contamination.

Odors used: 1-hexen-3-ol, trans-2-hexen-1-ol, cis-3-hexen-1-ol, 1-hexanol, 1-heptanol, 1-octanol, hexanal, heptanal, octanal, nonanal, 3,7-dimethyl-2,6-octadiene-nitrile, 3-pentanone,2-heptanone, 3-heptanone, 5-nonanone, 6-undecanone,cherry, mint.

2.4.3 Intracellular recordings

GGN

Sharp electrode recordings from large GGN process along the peduncle were made with borosilicate glass micropipettes (DC resistance, 60MΩ) filled with 3M K acetate. Input resistance was ~15MΩ*andtherestingmembranepotential* − 51.9± 4.9mV. GGN could be recognized by the presence of characteristic IPSPs during baseline and graded response to odor. A series of pilot experiments,in which the recorded cell was stained intracellularly by injection of 6% cobalt hexamine, lucifer yellow or

biocytin confirmed it always to have GGN's characteristic anatomy.

KENYON CELLS

Sharp electrode recordings from kenyon cell (KC) were always made from their somata (5-7 μm diameter) using borosilicate glass micropipettes (DC resistance, 250MΩ) filled with 0.5M K acetate.

β-LOBE NEURONS

Sharp electrode recordings from the dendrites of β-lobe neurons were also made with borosilicate glass micropipettes (DC resistance, 100MΩ) filled with 3M K acetate. Input resistance was around 300MΩ. The cell type from which the data are derived could be recognized by several characteristics, including response to odour, sub-threshold baseline activity profile, and response to electrical stimulation of Kenyon cells (Cassenaer and Laurent, 2007) . Recordings from β-LNs were always made from dendrites in the β-lobe (the largest dendrites are often several μm in diameter). That these recordings were not from Kenyon cell axons is guaranteed by the fact that Kenyon cell axons are too small for intracellular impalement (100–400nm diameter (Leitch and Laurent, 1996)). This identity of β-LNs was confirmed by dye injection.

2.4.4 Local Field Potential Recordings

Local field potentials in the calyx were always recorded in the mushroom body KC soma layer using Michigan probes. For simultaneous β lobe LFP recordings we used saline-filled patch pipettes (R_{DC}: 2–15 MΩ) or wire tetrodes.

LFP POWER MEASUREMENTS

We measured the average LFP power (in the 10-30 Hz band) as a function of time around an odor pulse. Power was calculated with a scrolling window (width 200 ms, step 50 ms) over all trials (ten per stimulus condition). Injecting current in GGN often caused an artifact so 150 ms on either side of the onset of current injection were eliminated from each trace (also in control) prior to power calculation.

KC POWER MEASUREMENTS

Similar to LFP power measurements expect the artifact was 10ms on either side of the current injection.

2.4.5 Electrical stimulation

Twisted-wire tetrodes obtained from FHC (number CE4B75) were modified for monopolar stimulation, with the casing serving as the anode. The tips of the tetrodes were splayed such that the distance between the exposed tips was approximately equal to 60% of the diameter of the mushroom body calyx. The exposed end of the stimulating electrode was embedded among Kenyon cell somata. The tetrodes were electroplated with gold solution to reduce the impedance to between 200 and 350 kΩ at 1 kHz. Stimulating currents (5–140 μA, 0.1 ms) were generated by an STG1000 Multichannel System.

2.4.6 Immunocytochemistry

Figure 1: We injected 5% biocytin in GGN for 1.5hr using 1Hz pulses at 5nA. We waited for 30min-1hr, desheathed the locust locust brain, fixed overnight in 5% formaldehyde and washed for 20 h in PBS. Brains were then dehydrated through

an ethanol series, placed in propylene oxide for 20 min, rehydrated and then agitated for five hours in PBS containing 5% triton and 0.5% bovine serum albumin (PBS 5% T 0.5% BSA). They were then washed for 30 min in PBS 0.5% T 0.5% BSA, and transferred to fresh PBS 0.5% T 0.5% BSA containing streptavidin-alexa488 (Invitrogen) at 1:100 dilution, incubated at 4°C for 2 days. Lastly, they were then washed for 30 min in PBS, dehydrated through ethanol series, cleared in methyl salicylate and examined by confocal laser scanning microscopy. Figure 1is a projection along the z-axis of a stack of 200 optical slices each 2 μm thick, constructed using the public domain *ImageJ* program (`http://rsb.info.nih.gov/ij/`).

Chapter 3

Drosophila GGN

3.1 Introduction

Our interest is in studying neuronal principles that can be generalized to other systems and modalities. In Chapter 1 I describe similarities in the organization of the olfactory system between vertebrates and invertebrates, and among different insect species. Here I specifically consider homology of the implementation of gain control in the mushroom body, using *Drosophila melanogaster*.

I have demonstrated in the locust (Chapter 2), the existence of a giant inhibitory neuron (GGN) whose role is to control the gain of the mushroom body, an area implicated in learning and memory (ref). This cell forms a feedback loop with the intrinsic cells of the MB, the Kenyon cells (KCs). Through this feedback loop it provides appropriate inhibition that is scaled to the total KC activity , which keeps the KC output within a dynamic range suitable for the downstream neurons. Furthermore I have shown that when we manipulate GGN's activity during odor to enhance or diminish it, we greatly affect the firing rate of neurons that are receiving input from KCs.

One reason for thinking this could be a generalizing principle, is that GGN

through this gain control maintains the sparseness of KCs in the locust. Recordings from KCs in *Drosophila* have shown these cells to represent odors sparsely as well (Turner et al., 2008; Murthy et al., 2008). So we asked whether in drosophila, as in the locust, there is a neuron that serves similar function?

In addition to addressing the generality of our findings in the locusts, an other important reason for looking for a potential homologue of GGN in the fly, is that it readily provides an opportunity to demonstrate a link between gain control and behavior. The importance of gain control as computational principle underlying many sensory and motor functions has been addressed extensively in the literature (Saalmann and Kastner, 2009; Robinson and McAlpine, 2009; Olsen and Wilson, 2008; Laurent, 1993). Furthermore, it is thought to mediate attention and underlie a special class of coordinate transformations (Salinas and Sejnowski, 2001; Reynolds and Chelazzi, 2004; Winkowski and Knudsen, 2006; Winkowski and Knudsen, 2008). Here we have a unique opportunity, by selectively and reversibly manipulating the activity of one neuron, to affect the output of a large neuronal population that is involved in olfactory processing and memory. Such a manipulation would provide a clear link between neuronal activity and behavior. The fly is a particularly attractive system for such an endeavor given the arsenal of genetic tools and the well-established olfactory behavioral assays that have already been developed (Benzer, 1967; Hotta and Benzer, 1970; Brand and Perrimon, 1993; Kitamoto, 2001; Quinn et al., 1974; Tully and Quinn, 1985; Pitman et al., 2009).

If the fly GGN exists and serves a function homologous to its locust counterpart, we would need to generate a line specifically targeting this cell; by using optogenetic(Boyden et al., 2005; Han and Boyden, 2007; Zhang et al., 2007a) and

other tools (Kasuya et al., 2009; Lima and Miesenbock, 2005; Pulver et al., 2009), we would attempt to manipulate its activity during a behavioral assay to assess the importance of gain control. In the locust we have demonstrated that enhancing GGN activity during odor has a powerful effect on the output of the MB, completely silencing downstream neurons. We could ask, then, what would happen if, using genetic tools, we manipulate GGN activity during odor in a behaving animal.

Here I discuss evidence that this cell exists in the fly; I also describe our attempts at generating a line that selectively labels GGN, as well as future behavioral experiments. Finally, I discuss a recently published study of the drosophila GGN.

3.2 Results & Discussion

Evidence for the existence of fly GGN, came from Greg Jefferis' work then at Liquon Luo laboratory (Jefferis et al., 2001). He used the MARCAM technique to generate single PN clones of the GH146 line and study what dictates a PN's targeting of a glomerulus (Jefferis et al., 2001). While most single clones labeled PNs (Fig 1), in one such experiment a cell with processes throughout the MB and none in the AL was labeled. Based on the similarities with the locust GGN anatomy, we hypothesized that this cell could be the GGN equivalent in the fly. In figure 2 we present both image stacks for comparison.

One of the anatomical differences is that GGN in the locust sends projections to the α lobe only, whereas in the fly the processes seem to extend to all lobes. One potential reason for that difference is that in the locust all KCs bifurcate to to send axons in the α and β lobes (Laurent and Naraghi, 1994). Therefore it appears potentially redundant for GGN to send processes in both the lobes and sample the same KC output. Conversely, in the fly there are KCs that send projections solely to

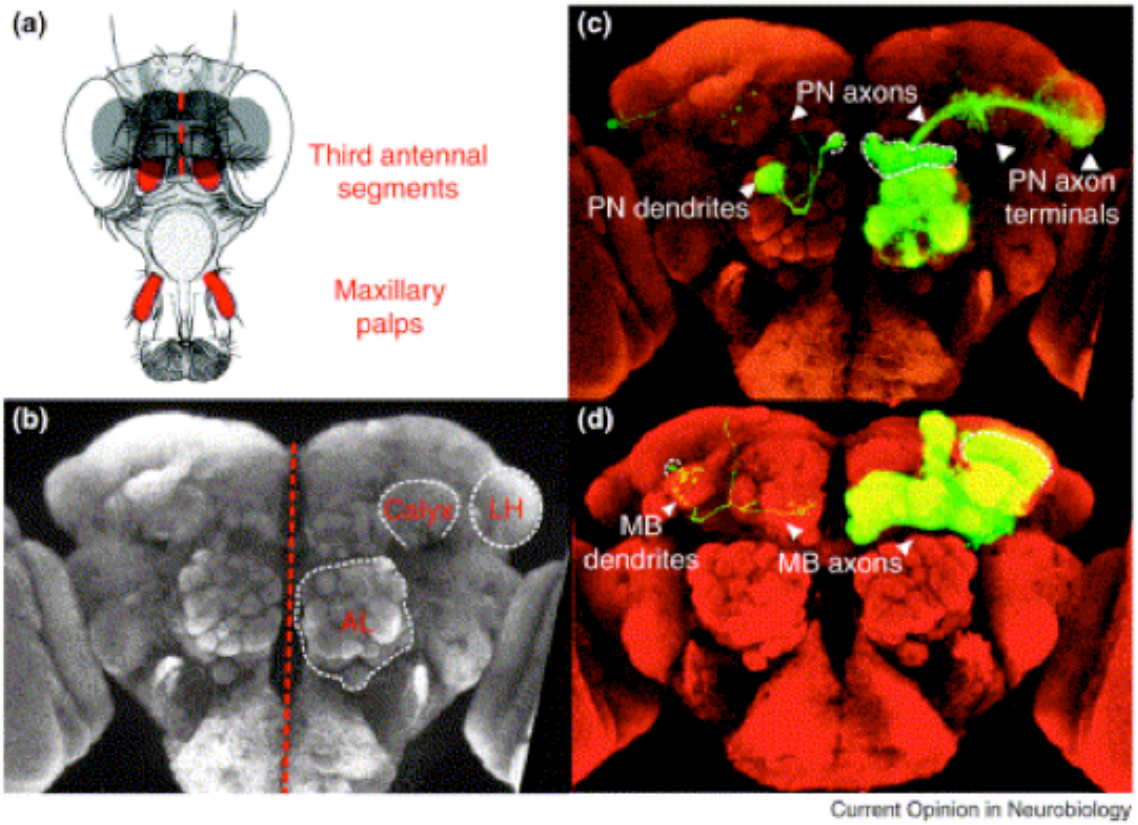


Figure 3.1. Visualising PNs and MB neurons. AL, antennal lobe; LH, lateral horn; MB, mushroom body; **A.**Schematic of a Drosophila head (anterior view) with the olfactory appendages marked in red and the outline of the brain in dark grey. **B.**A brain in the same orientation stained with the monoclonal antibody nc82, which recognises neuropil. Note the position of the AL, the calyx of the MB (Calyx) and the LH. The midline is indicated with a dotted red line. **C.**The same brain as in (B) showing, in green, an anterodorsal PN single cell clone on the left and an anterodorsal neuroblast clone on the right; nc82 stained neuropil now pseudocoloured red. Cell bodies are outlined by dotted lines. (D) An MB single cell clone of the α'/β' type (left) and a neuroblast clone (right). The image is a composite of two original confocal stacks. Adapted from Jefferis *et al.* (2002)

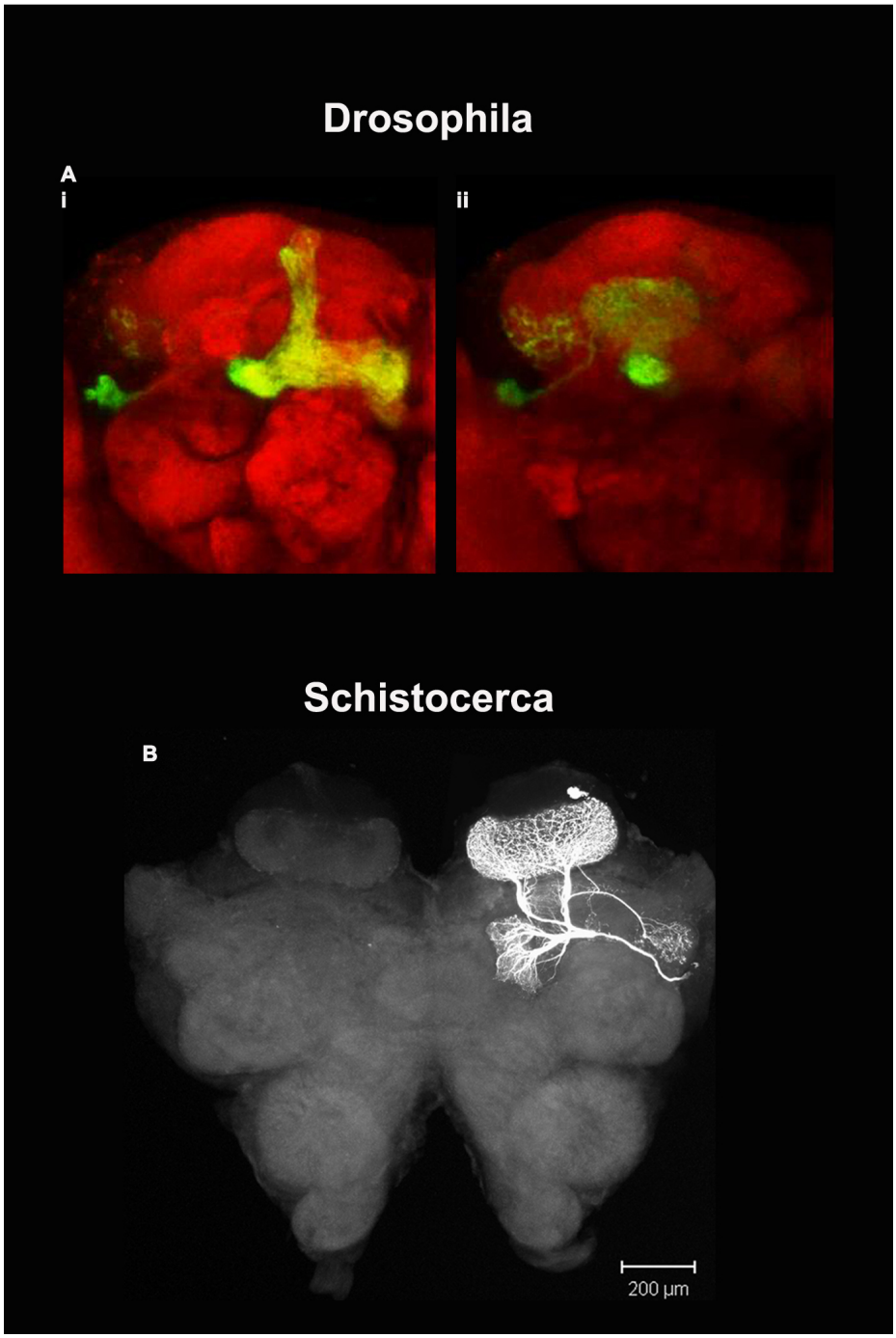


Figure 3.2. Anatomy or Presumed GGN in drosophila & locust GGN. **A.** Image stacks of a GH146-Gal4 UASGFP MARCM clone, showing extensive processes in the mushroom body lobes (Ai), calyx & lateral horn (Aii) of a cell with a large cell body in the lateral horn (Ai&Aii). **B.** Image stacks of an intracellularly filled GGN with 5% biocytin, showing also extensive arborization in the mushroom body. Cell body lightly stained in the lateral horn (biocytin injection site along a process in the peduncle).Image in A courtesy of Greg Jefferis.

the α & β lobes, α' and β' lobes, or the γ lobe (Crittenden et al., 1998). Given these three different classes of KCs would appear to be necessary to sample all lobes in the fly.

3.2.1 What makes this neuron a homologue of the locust GGN?

In the locust, as described in Chapter 2, I have shown that GGN is an inhibitory neuron that receives input from KCs and feeds back onto KCs to scale their activity according to the total KC activity. I have further shown this neuron to be non-spiking, to respond to odors with a graded potential, and to increase its membrane depolarization (& therefore inhibition onto its targets) with an increase in odor concentration. I next discuss evidence that in drosophila the above neuron shares most of these characteristics and we therefore refer to it as drosophila GGN (dGGN).

First I sought to determine whether dGGN was also GABAergic. Using the GH146-Gal4 line, we drove UAS GFP in all the cells labeled by this line, including GGN. GGN can be distinguished by other cells labeled by this line by its big cell body located in the lateral horn. Immunocytochemical staining with an antibody against glutamate decarboxylase (GAD), a key enzyme in the GABA biosynthesis, co-locolalized with GFP in the large cell body, showing this neuron to be GABAergic.

Secondly, we were interested in evaluating this cell's odor-response.

Due to technical considerations, in terms of accessibility and location of the cell body, I could not perform patch clamp recordings. To achieve this task, I therefore recorded intracellularly with sharp electrodes from this cell in the fly *in vivo*. To target the cell, we crossed the GH146-Gal4 line with a UAS GCamp line. Gcamp is a calcium indicator that fluoresces upon binding to calcium (Nakai

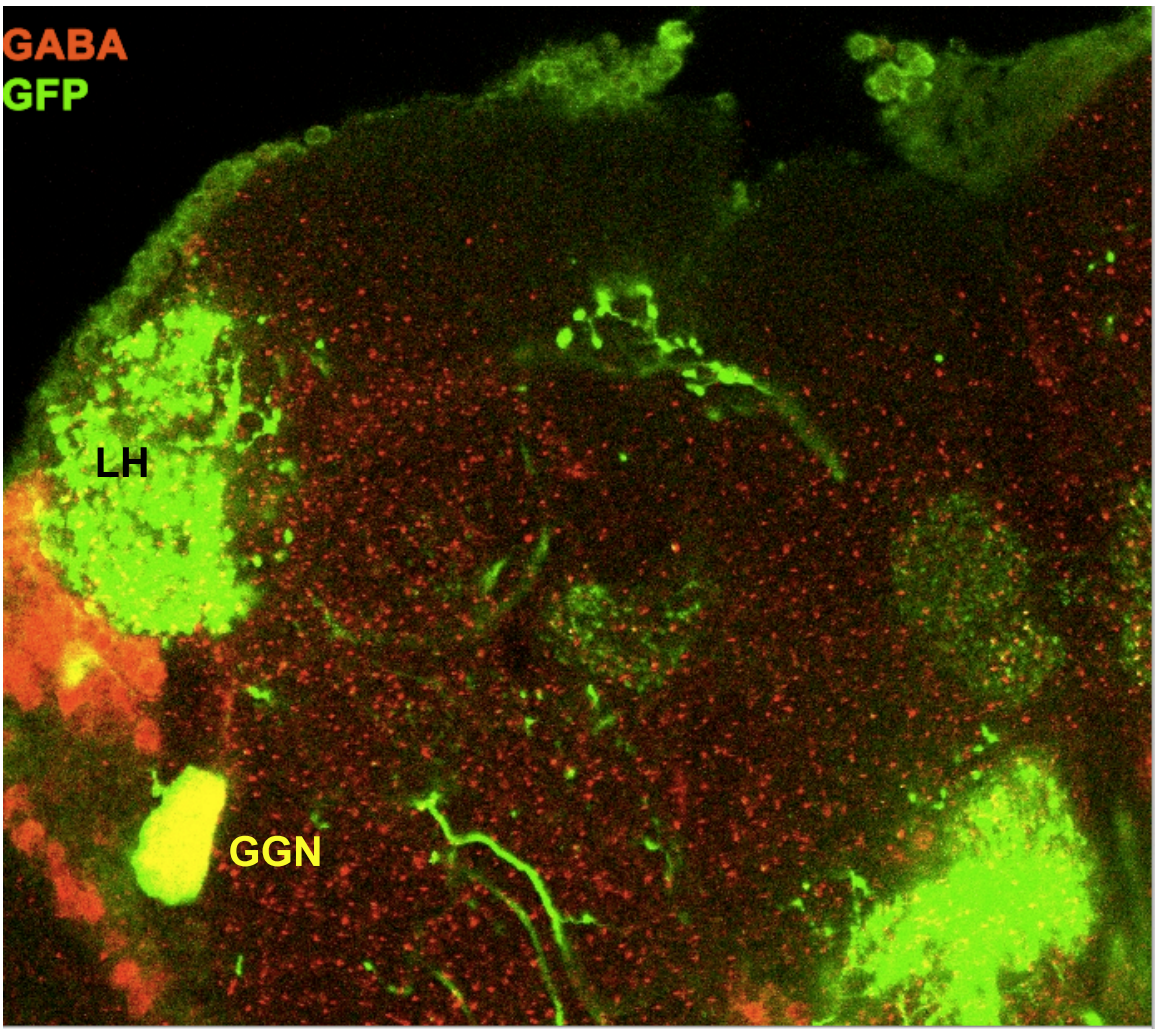


Figure 3.3. Drosophila GGN is GABAergic Image showing double labeling in drosophila GGN cell body for GABA (red) and GFP (green) using immunocytochemistry. UAS GFP is driven by the GH146-Gal4 line & iand the signal is amplified through an antibody against GFP. GABA is detected by an antibody raised against GAD. LH: lateral horn

et al., 2001). Within a certain PN firing rate range, this change in fluorescence has been shown to correlate well with PN firing (Jayaraman and Laurent, 2007; Tian et al., 2009). I took advantage of this observation in order to determine the intactness of our prep. Specifically, using this line, we looked for a change in fluorecence (δF) in response to an odor puff in PNs , which are also labeled by the GH146 line. We used PNs rather than GGN because (with this indicator) we could not observe an unambiguous δF signal in the GGN soma in response to an odor. However, baseline fluorescence was sufficiently high to be used as a guide for the intracellular electrode to the dGGN cell body located in the lateral horn.

The membrane potential change in drosophila GGN in response to an odor appeared very similar to it's locust homologue. Namely, the cell responded with depolarization of a similar time course to its locust counterpart. In addition, dGGN also appears to be non-spiking, as we never observed any spikes in response to either odor-stimulation or intracellularly injected depolarizing current. IPSPs and spikelets are not as prominent in the fly GGN as in the locust, but this most likely reflects differences in recording location between the two species rather than a biological difference. The filtering properties of the soma could easily account for the attenuation of fast changing signals observed in fly GGN vs recordings in the locust from processes that tend to be closer to the input or output. In order to address this issue one would need to record intracellularly from a GGN process in a similar location in the fly.

Further proof for the fly GGN being homologous to the locust GGN came from intracellular GGN recordings to increasing odor concentrations. One characteristic feature of the locust GGN is the increase in the DC component of GGN odor response with an increase in odor concentration. This proved to be true also for

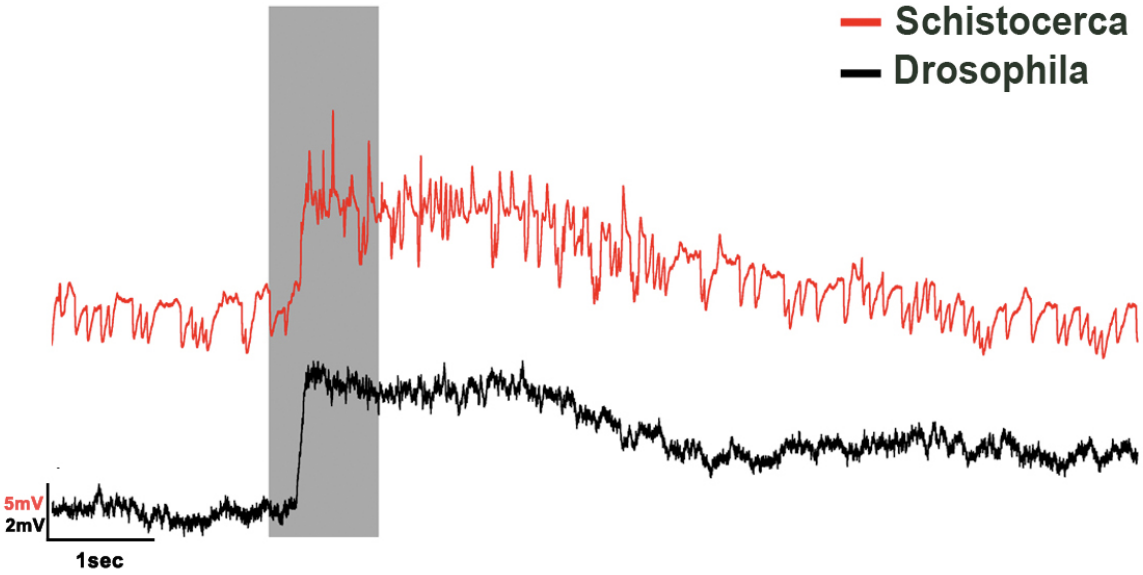


Figure 3.4. Drosophila and locust GGN Odor Response. Intracellularly recorded membrane depolarization in response to an odor in locust (red) and drosophila GGN (black) presented offset for display purposes. Odor bar: odor on.

fly GGN, suggesting that dGGN also provides increased inhibition in response to increasing odor concentration, , and presumably also serves to partially counteract a concomitant increase in KC activation.

I have shown that locust GGN receives input from KCs, and indirect observations suggest that this is the case also for dGGN. In particular, we observed that dGGN receives input in the MB lobes, presumably from KCs. This was determined by imaging the GH146-Gal4 line, here used to drive expression of UAS synaptofluorin (spH) . Synaptofluorin is targeted primarily to the release sites of neurons (Miesenbock et al., 1998; Ng et al., 2002; Miesenbock, 2004). In the aforementioned fly line we observed bright staining in the calyx[1], where KCs receive input. In contrast, the signal in the lobes was very weak (3.6), suggesting that the GGN

[1] As well as in the LH and in the AL.

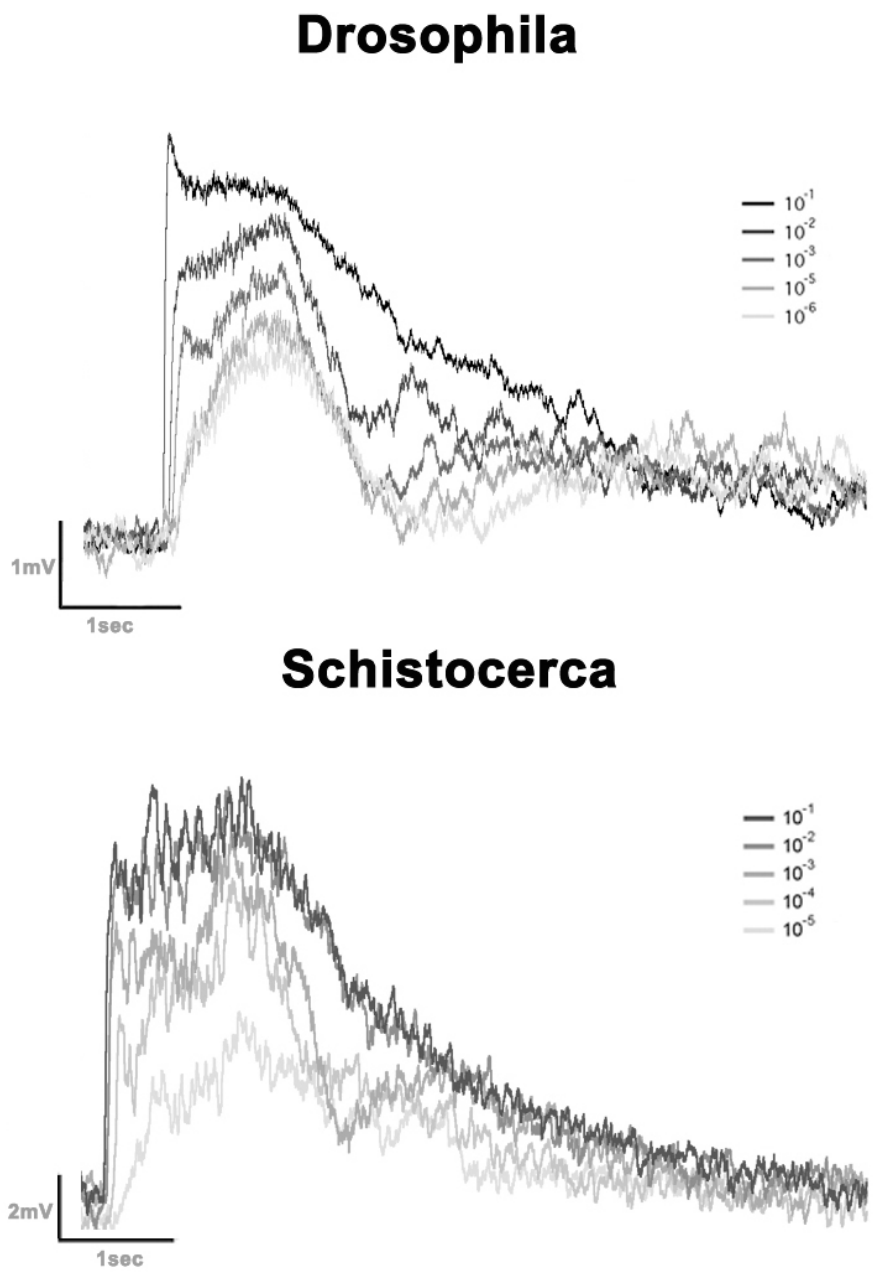


Figure 3.5. Drosophila and locust GGN Response to Increased Odor Concentration. Intracellularly recorded membrane depolarization in response to a series of odor concentrations in locust (right panel) and drosophila GGN (left panel). color coding: light to dark grey represents increase in odor concentration. dGGN impaled in the soma, locust GGN in a process along the peduncle. Odor duration: 1sec.; Odors: benzaldehyde (drosophila), octanol (locust).

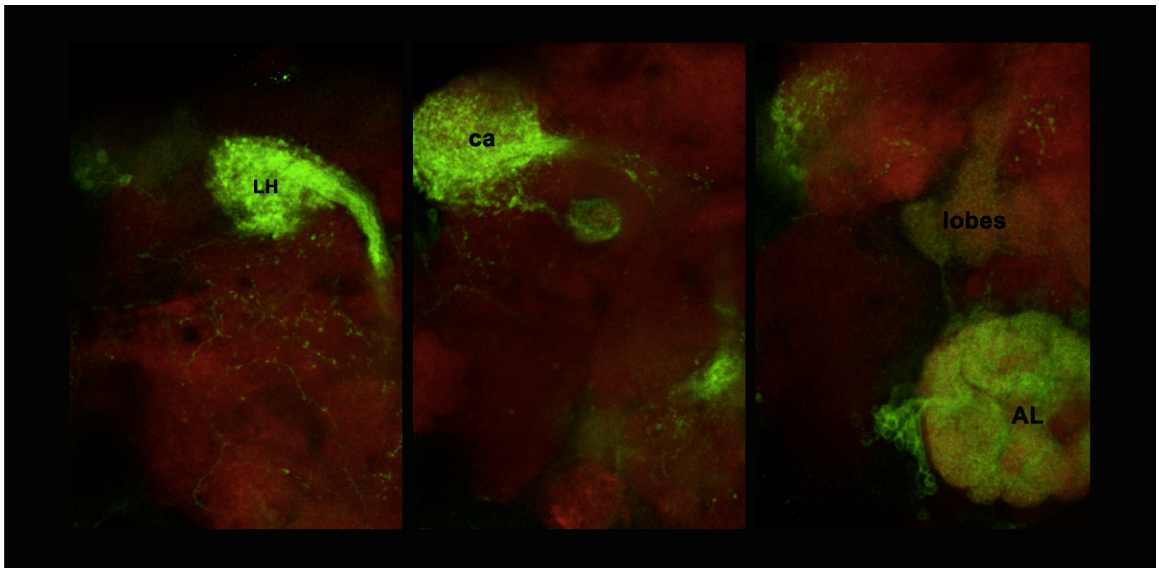


Figure 3.6. Imaging output synapses in GH146-Gal4 with UAS Synapto-Phluorin. Images shown were taken from the same Z stack of images with the same confocal settings. Strong labeling in the lateral horn (LH) and mushroom body calyx (ca) shown in left and middle panels respectively. Labeling in the antennal lobe (AL) indicative of some output from PN processes in the AL glomeruli. Very faint labeling in the lobes consistent with this area being mostly an input zone. Compare with GFP labeling shown for GGN in figure 2.

processes shown to be located in that neuropil receive input from KC axons there[2]. The significance of the difference between the lobes and the calyx (and LH) of the UAS SpH flies is underscored by the stacks showing the same regions all labeled strongly in the single GGN clone combined with UAS GFP (Figure 3.2).

In summary, I have shown that the anatomy, odor response, neurotransmitter (GABA) and increased inhibition to increased odor concentration are common between these two species, suggesting that GGN is a general solution for providing gain control for the MB, at least across these two species. An additional goal for identifying dGGN, was the hope that we could take advantage of fly genetics, which together with molecular tools could allow us to manipulate GGN activity in an intact freely behaving animal. As such we could test the link between changes

[2]Although, it should be noted that in insects input and output is not always clearly defined; sometimes they can be intermixed.

at the network level with behavioral changes.

3.2.2 Generating a specific GGN line

As mentioned previously, the GH146-Gal4 line that labels GGN, also labels a large fraction of PNs (Stocker et al., 1997; Jefferis et al., 2001). Therefore any manipulation of GGN that would take advantage of the UAS GAL4 system would also inadvertently affect PNs and consequently compromise the results. For that purpose, I sought a genetic strategy to limit expression to GGN.

Our approach was to take advantage of the fact that PNs are cholinergic(Yasuyama et al., 1996; Yasuyama et al., 2002; Yasuyama et al., 2003) while GGN is GABAergic and use a suppressor of the GAL4 transcription factor (GAL80) to be expressed selectively in cholinergic cells (Kitamoto, 2002). This suppressor would be driven by the cholinergic promoter and would therefore -in principle at least- only be active in cholinergic cells, rendering the GAL4 transcription factor in these cells inactive. In such a scenario, GAL4 expression should be limited to GGN and should therefore allow us to restrict UAS expression to GGN.

Unfortunately, this strategy did not prove successful, probably due to the "leakiness" of the promoter driving GAL80 expression. Imaging the aforementioned flies showed no signal in GGN, suggesting that GAL80 was suppressing GAL4 expression outside of cholinergic neurons as well. An additional issue with this approach was that a careful examination of the PNs targeted by this line, yielded a small number that are GABAergic (Shang et al., 2007). It is therefore clear that a different targeting approach would be required to generate a GGN-specific line.

3.3 Future experiments:

3.3.1 Closing the feedback loop; GGN output on KCs during odor

Imaging & Physiology

The evidence for the homology of these two species is strong. To conclusively demonstrate that indeed the fly GGN operates analogously to its counterpart in the locust we want to combine 2-photon imaging with electrophysiology. Specifically, we want to manipulate GGN activity intracellularly during odor while imaging KC activity in the somata with GCaMP3 (Tian et al., 2009) driven by GH146-Gal4 line. I expect that enhancing GGN activity during odor would lead to a decrease in KC activity, which could be imaged across KC somata. For these experiments, 2 GAL4 lines would be combined: the GH146 line to label GGN and the OK107 line to target a large number of KCs. GGN's activity would be manipulated with an intracellular electrode, while GCamp fluorescence would be used to determine the effect across many KCs simultaneously. The imaging would take place in KC somata to avoid any bleed through effect from PN & GGN processes in the calyx. The sensitivity of the latest version of GCaMP3 is considerably enhanced over previous versions (Tian et al., 2009) which would allow us to detect changes in fluorescence resulting from sparse KC spiking.

3.3.2 Behavioral assessment of GGN's gain control in olfaction

Behavioral experiments:

Following characterization of the dGGN effect on KCs, I will assess the behavioral effect of dGGN manipulation on olfactory learning and memory. In the locust, we have shown that by enhancing GGN's activity during odor we can essentially

shut down the β lobe neurons that receive input from KCs (Chapter 2). The synapses made by KCs onto this class of extrinsic MB neurons are governed by STDP, which synchronizes the β-LN population by maintaining their firing at a particular phase of the LFP oscillation during the odor response (Cassenaer and Laurent, 2007). Recent electrophysiological recordings have demonstrated that in the presence of the neuromodulator octapamine, the STDP curve changes shape, leading to a depression of KC- β-LN synapses that are active during the odor (Cassenaer and Laurent, in preparation). Neuromodulators, are thought to mediate aversive and rewarding stimuli and behavioral experiments suggest that they act in the mushroom body(Davis, 2005). The electrophysiological evidence mentioned provides a framework for thinking about how rewarding (and probably aversive) stimuli can act by selectively affecting the relevant KC synapses onto this extrinsic neuronal population to mediate learning (Cassenaer and Laurent, in preparation). I would therefore expect that a manipulation that can shut these neurons down would greatly affect associative learning. This approach is a unique opportunity to affect the output of all KCs and assess in a reversible way the effect of greatly reducing KC activity. Importantly, it allows us to highlight the importance of gain control in this system and the behavioral consequences of eliminating it in a reversible way.

Critical for the success of these experiments is the generation of a line that specifically targets GGN. We are in the process of obtaining such a specific line for GGN. To activate GGN we could use optogenetic tools such as channel rhodopsin[3] or alternatively tools ie the P2X2 receptor(Lima and Miesenbock, 2005; Claridge-Chang et al., 2009). Calcium imaging and electrophysiology will be used to assess the effectiveness of dGGN activation by ATP-mediated P2X2 activation. In the

[3]In the fly, blue light is required for activation of the channel; flies show a strong response to this light alone, so we are less likely to use this approach

final step, we plan to manipulate dGGN by activating the P2X2 channel during olfactory learning. We expect that flies in which GGN is selectively activated during olfactory learning with a positive reinforcer will not form memories. This could be assessed in a T-maze assay in an olfactory appetitive conditioning paradigm, where one of two odors is paired with sucrose (Tempel et al., 1983; Keene et al., 2006; Krashes et al., 2007; Pitman et al., 2009). Finally, while we focused on appetitive conditioning because of octopamine's involvement in reward, we expect aversive conditioning to be equally affected.

3.4 Comparison with recently published result

In this chapter we have provided evidence based on anatomy and physiology that strongly suggest the identified neuron to be a homologue of the locust GGN. We now turn to a study published recently that identified this neuron, confirming our anatomical results, and suggested a function for this neuron in olfactory learning and memory that differs from our interpretation. (Liu and Davis, 2009). The authors named this neuron based on its cell body location: the anterior paired lateral neuron (APL). To avoid confusion we will continue to refer to it as dGGN when describing their results.

To isolate this neuron, similarly to Greg Jefferis' approach, the authors of this study generated ~300 single clones of the GH146-Gal4 line using MARCM analysis (Jefferis et al., 2001). Using the same approach as ours shown in figure 3, the authors confirmed the cell's GABAergic nature through antibody detection against GAD. In order to restrict expression of this GAL4 line to just GGN, they attempted to use the ChaGal80 suppresor, and, consistent with our results, they observed that the suppressor's activity was not limited to just cholinergic cells but seemed to

also affect GGN. Another strategy that proved more fruitful was to use RNAi for GAD in the GH146-Gal4 line. By performing this manipulation, they observed a reduction in the GABA present in dGGN as compared to control GABAergic neurons in the ellipsoid body of the central complex. Using this line for behavioral experiments, they observed that this manipulation enhanced olfactory memory. Conversely, they observed that if they drove the GAD RNAi in the ChaGal80 GH146 flies where the GAL4 expression in GGN is also eliminated, the authors could not observe any enhanced learning. Therefore the authors concluded that this learning enhancement effect observed in the GH146-Gal4 line with the GAD RNAi is most likely mediated by GGN and therefore GGN's main role is to inhibit learning.

There are several concerns about this approach. Firstly, as acknowledged by the authors, there are other GABAergic neurons also targeted by the GH146-Gal4 line. The main results of the paper are very likely influenced by the joint manipulation of those neurons (Shang & colleagues counted ~7.5 GABAergic neurons in the AL of the GH146-Gal4 line,(Shang et al., 2007)).

The number of inhibitory PN neurons that are affected is small but could affect the larger antennal lobe network and could further complicate the interpretations of such an experiment; PNs excite lateral interneurons in the antennal lobe, which in turn provide widespread inhibition to most if not all glomeruli. It is unclear how these inhibitory PNs fit in this scheme, but given that activity tends to spread across the AL , changes in the activity of a few PNs could easily propagate across the network and subsequently to KCs and extrinsic MB neurons. Furthermore, given that GAD RNAi is expressed during development, it is quite possible that such a manipulation could influence the development of the network (e.g. change

connection strengths) and its connectivity and place it in a very different state than in the unaffected animal.

Lastly, the authors report changes in dGGN's response to an odor -as assayed by calcium imaging- after pairing the odor with an electric shock. This is a very interesting observation and, if true, would have implications for modifying GGN-mediated gain control in the MB. However, some cause for concern is the existence of additional GABAergic cell bodies in the LH that are also targeted by the GH146-Gal4 line (Figure 7). In the locust, β-LNs are GABAergic cells that have cell bodies located in the LH and processes in the MB lobes (MacLeod and Laurent, 1996; Cassenaer and Laurent, 2007). [4] Based on recent electrophysiological evidence we known that β-LN activity is altered in response to an odor paired with a neuromodulator[5], which should be reflected in the output in the lobes onto other β-LNs . Therefore, when imaging with SpH in the horizontal lobe in this Gal4 line, it would be unclear whether the activity recorded is due to GGN or to other GABAergic neurons that also project in the lobes.

In summary, this study confirms our preliminary anatomical results, provides some support for the role for GGN within the olfactory circuit, but also highlights the importance of temporal and spatial control of any manipulation in order to determine accurately the cell's role in olfaction.

[4] These cells receive input from KCs and also provide inhibition to neighboring β-LNs, presumably also in the lobes (Cassenaer and Laurent in preparation).

[5] Neuromodulators have been identified as mediating aversive and appetitive stimuli.

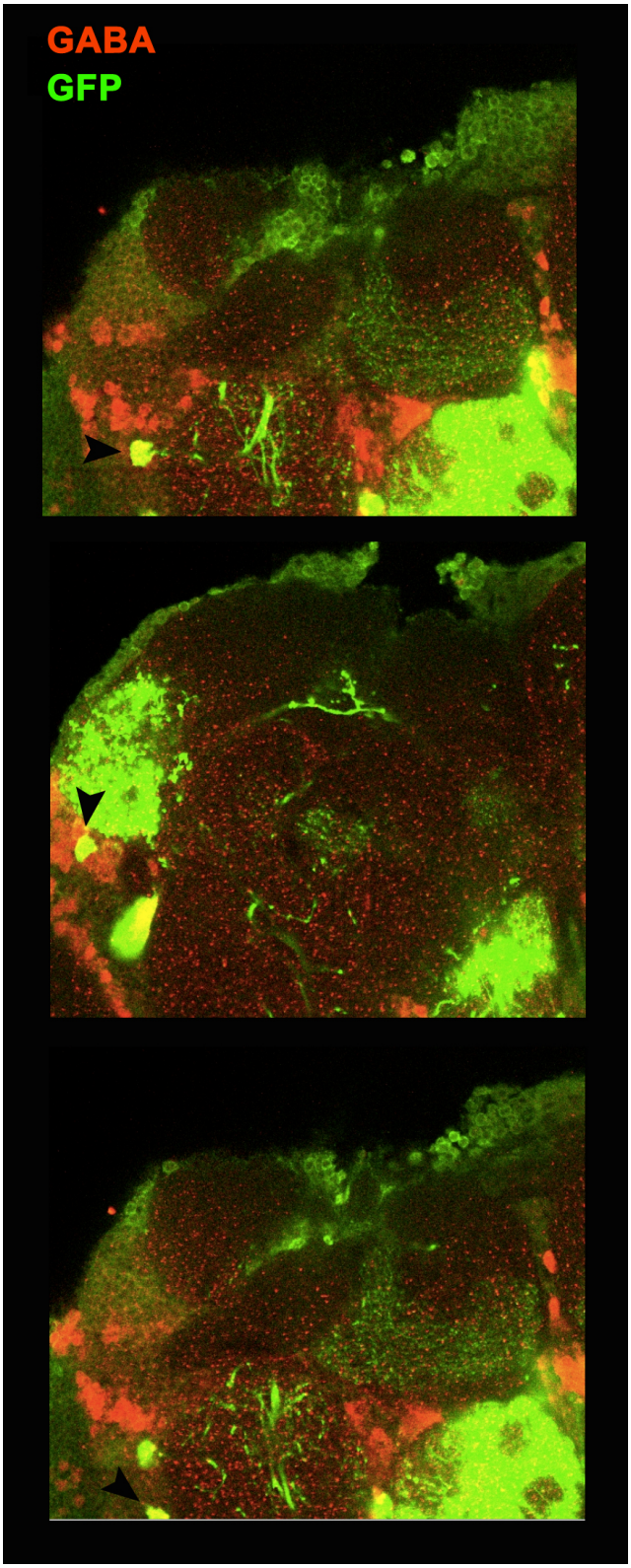


Figure 3.7. Additional GABAergic labeled GH146-Gal4cells. Three additionally double labeled cells in drosophila for GABA (red) and GFP (green) using immunocytochemistry. UAS GFP is driven by the GH146-Gal4 line & iand the signal is amplified through an antibody against GFP. GABA is detected by an antibody raised against GAD. Arrowheads point at each example.

3.5 Methods

3.5.1 Fly lines

Flies were reared on standard cornmeal agar medium. We used the Gal4/UAS-system (Brand and Perrimon, 1993) to direct the expression of the calcium sensor G-CaMP (Nakai et al., 2001) to specific cells. GH146-Gal4 flies were a gift from L. Luo (Stanford University, Stanford, CA). UAS-GCaMP flies were a gift from R. Axel (Columbia University, New York, NY). All experimental animals were adult females, 2-3 days after eclosion.

3.5.2 Fly preparation

Flies were anesthetized in a glass vial on ice just until movement stopped (20 sec.), and then were gently inserted into a hole in a piece of aluminum foil. Small drops of wax (55ąC) were used to suspend the fly in the hole, with the edge of foil defining a horizontal plane around the head and thorax, from the first antennal segment anteriorly to the scutellum posteriorly. The dorsal side of the foil was bathed in saline, while the ventral side (including antennae and maxillary palps) remained dry and accessible to odors. A window was cut in the dorsal head cuticle between the eyes, extending from the ocelli to the first antennal segment. Fat and air sacs dorsal and anterior to the brain were removed, and the perineural sheath was gently picked away from the antennal lobes. The proboscis was affixed with a small drop of wax to a strand of human hair to limit brain movement. Spontaneous leg movements were typically observed in this preparation for the duration of the recording (1.5-3 hour). The saline composition used in all experiments was (Wang et al., 2003): 108 mM NaCl, 5 mM KCl, 2 mM CaCl2, 8.2 mM MgCl2, 4 mM NaHCO3, 1

mM NaH2PO4, 5 mM trehalose, 10 mM sucrose, 5 mM HEPES [pH 7.5, 265 mOsm].

3.5.3 Odor delivery

Odors (benzaldehyde (ba),isoamyl acetate (ia), 1-hexanol (hex), eugenol (eug), acetophenone (ace), citral (cit), cherry (che)) were delivered using a custom-made odor-delivery system and a Teflon nozzle (entry diameter 1 cm, exit 0.1 cm) directed towards the antennae. Odors were delivered in a constant stream of air (0.4-0.8 l/second) at final concentrations of ca. 0.5-50%. Odors were diluted 10% volume/volume in paraffin oil. Different odor concentrations were prepared by serial dilutions, ie 10^{-2} is 1:100 dilution of odor in paraffin oil.

3.5.4 Electrophysiology

Sharp electrode recordings from GGN were always made from its soma that was identified by GCaMP baseline fluorescence in the GH146 Gal4 line. Electrodes were made using borosilicate glass micropipettes (DC resistance, 100-150MΩ) filled with 3M K acetate and 0.1 mM sulforhodamine B (Molecular Probes, Eugene, OR). Upon GGN impalement with the electrode, the sulforhodamine diffused into the soma, giving me confidence I was recording from GGN. Current data were acquired via an Axoclamp-2B amplifier (Axon Instruments, Union City, CA), a National Instruments A-D card (15 kHz sampling) and LabView software (National Instruments, Austin, TX).

3.5.5 Immunohistochemistry

For anti-GABA staining of brains that also express a CD8GFP cell marker, brains were fixed for 15 min in 5% formaldehyde in PBS, rinsed with PBS, and blocked in 5% normal goat serum/PBST (0.2% Triton X-100 in PBS) for 20 min. Brains were incubated in 1:100 rabbit anti-GABA antibody (catalog A2052; Sigma, St. Louis, MO) and 1:100 rat anti-GFP antibody at 4^{o}C for 2 d. After washing for 1 h in several changes of PBST, brains were incubated with 1:250 goat anti-rabbit:Alexa Fluor 568 and 1:250 goat anti-rat:Alexa Fluor 488 (Molecular Probes, Eugene, OR) for 2 d at 4^{o}C . To visualize neuropil along with the UAS SpH flies, brains were incubated in 1:10 mouse nc82 antibody (gift from E. Buchner, University of Wurzburg, Wurzburg, Germany) for 24 h at 4^{o}C and then washed for 30 min in several changes of PBST before incubation with 1:1000 goat anti-mouse:Alexa Fluor 568 and 1:1000 streptavidin: Alexa Fluor 488 for 24 h at 4^{o}C . After incubation with secondary detection reagents, brains were washed for 20 min in PBST and mounted in Vectashield on a slide flanked by two 1 coverslips. Confocal fluorescence microscopy was performed on a Zeiss (Oberkochen, Germany) LSM 510 using a 20x objective.

Chapter 4

Additional GGN Features

4.1 Introduction

In Chapter 2, I discussed evidence that GGN provides necessary gain control in the mushroom body (MB) of the locust, by means of a negative feedback loop between GGN and Kenyon Cells (KCs). In this chapter I examine other aspects of GGN that contribute to or further illustrate its function. First, I discuss experiments that are directly related to input to GGN. I showed previously that the GGN membrane potential tracks the local field potential (LFP) during the odor response and I was therefore interested in assessing whether GGN receives direct input from projection neurons. I also discuss the discovery of a form of short term facilitation at KC-to-GGN synapses and potential reasons for its existence, as well as a description of alternative ways to assess GGN's function in this circuit. In Chapter 2, I described the effect of manipulating GGN during odor stimulation on the LFP recorded in the MB calyx. Given that the LFP reflects better the input into an area rather than the output, we chose to record the LFP in the β lobe in order to get a sense of the effect of this manipulation on KC output. Lastly, I show data that assess the effect of GGN ablation on KCs.

4.2 Results & Discussion

As described in Chapter 2, in order to assess KC input onto GGN, we used an extracellular stimulating electrode placed in the KC soma layer. A single stimulation pulse was shown to result in a post-synaptic potential (PSP) onto GGN. Furthermore, we showed that when we stimulated a group of KCs at the LFP frequency (that is the predominant frequency at which KCs receive input from PNs) we were able to reproduce the DC component of the response (Chapter 2, Fig 3), illustrating that GGN's relatively long time constant allows the inputs to be summated, giving rise to this DC shift.

KINETICS OF GGN RESPONSE TO KC STIMULATION CORRELATES WELL WITH ODOR RESPONSE IN THE SAME LOCATION

Further support for the fact that KC input shapes GGN odor response comes from an experiment where a stimulation electrode was placed in the KC soma layer and GGN was impaled with an intracellular electrode in 3 different subsequent locations along the electrode path. As discussed, GGN's response to odor is characterized by a DC component with spikelets riding on top. The width of the spikelets appeared to depend on the location of impalement. We assume that this would be a function of the distance from the cell's regions of input/output, as well as the density of voltage gated channels. We observed that the width of the odor-evoked spikelets correlated well with the width of the events observed in response to KC stimulation, providing a further link between KC activation and GGN odor response.

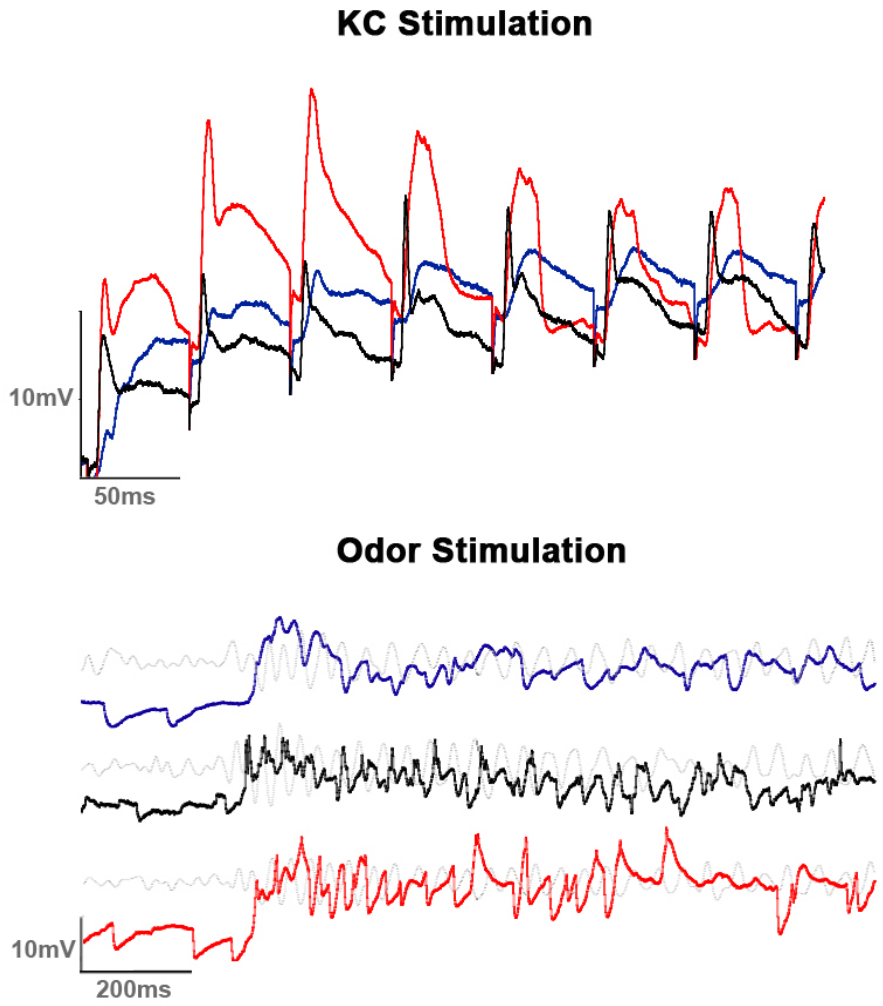


Figure 4.1. Kinetics of GGN's response to KC & Odor Stimulation in 3 locations along GGN.Top panel shows GGN's response to a train of KC stimulation (50ms apart) recorded in 3 different GGN locations consequetively in a single penetration. Shown in blue, a four trial average membrane potential response to KC stimulation recorded in the most superficial GGN location (Stimulation strength: 20uA). Shown in black is a four-trial average membrane potential response to KC stimulation recorded in a GGN location with observed (unusual [1]) excitatory events during baseline (Stimulation strength: 10uA).Shown in red, a nine trial average membrane potential response to KC stimulation recorded in a usual GGN recording location. Bottom panel showing examples of single trial odor response to 10% octanol as recorded in the above 3 locations with corresponding bandpassed 10-30Hz LFP (grey). Note similarities in the width of excitatory potentials over both conditions.

4.2.1 *Facilitation*

In one experiment we observed that the response of GGN to a single KC stimulation pulse increased after repeating many trials of trains of KC stimulation. While such a train of KC stimulation might not be physiologically relevant [2], we were interested in investigating whether this initial observation might be indicative of something more relevant. Given that KCs responding to an odor typically fire approximately two spikes at an average interval of 200ms (Perez-Orive et al., 2002), we repeated the stimulation experiment with two KC stimulation pulses separated by 200ms. In these experiments, we observed short term facilitation: the amplitude of GGN's response to the 2nd pulse was increased compared to the first.

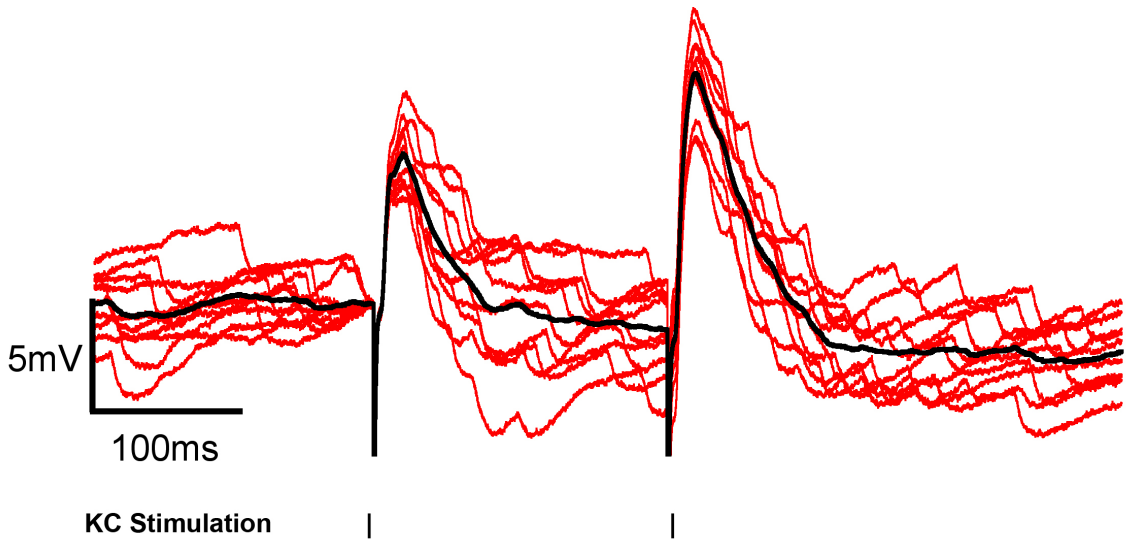


Figure 4.2. Facilitation of the KC to GGN synapse.Example of the facilitating postsynaptic effect a KC stimulation pulse has on GGN for a subsequent pulse of equal magnitude. Interval between pulses 200ms.

Next we tried to assess to what extent this increased GGN response was sensi-

[2] most KCs when they respond to an odor, they fire only a couple of spikes and here we stimulated the same group of KCs for an average of 20 pulses per trial for ~ 50 Trials

tive to the timing between KC spikes, by varying the stimulation interval. Below is an example of one such experiment, followed by a curve summarizing results from such averages of 4 experiments. The results presented are ratios of maximum amplitude in response to the 2nd pulse divided by the response to the first pulse. Maximum amplitude is calculated by finding the peak in the response and subtracting the value at the foot of the PSP. If there is no change in the GGN response to those two KC stimulation pulses, then this ratio should be 1. Larger than 1 implies the response to the second pulse was increased over the 1st pulse. We observe that there appears to be a general facilitation for intervals of 1s and smaller, with a peak at 50ms & 250ms.

We propose that such facilitation could play various roles. First it could mediate what effectively amounts to lateral inhibition among KCs. Through this short term facilitation the impact of a KC on GGN could be amplified with a second spike and therefore through GGN inhibition increase the threshold for firing of other KCs that did not yet have a chance to fire at this cycle. Additionally, this facilitation could serve to boost GGN's activity, to overcome the inhibition from the neuron described in Chapter 2, because of the fact that most KCs, when responsive to an odor, fire two spikes in quick succession (on average ~200ms). As we have seen, this inhibitory cell, like GGN likely receives input from KCs during odor and inhibits GGN. GGN in turn provides inhibition back onto the cell. One hypothesis, then, is that by means of facilitation, GGN takes advantage of the doublet KC firing during odor, to specifically amplify its influence only in the presence of odor and not at baseline.

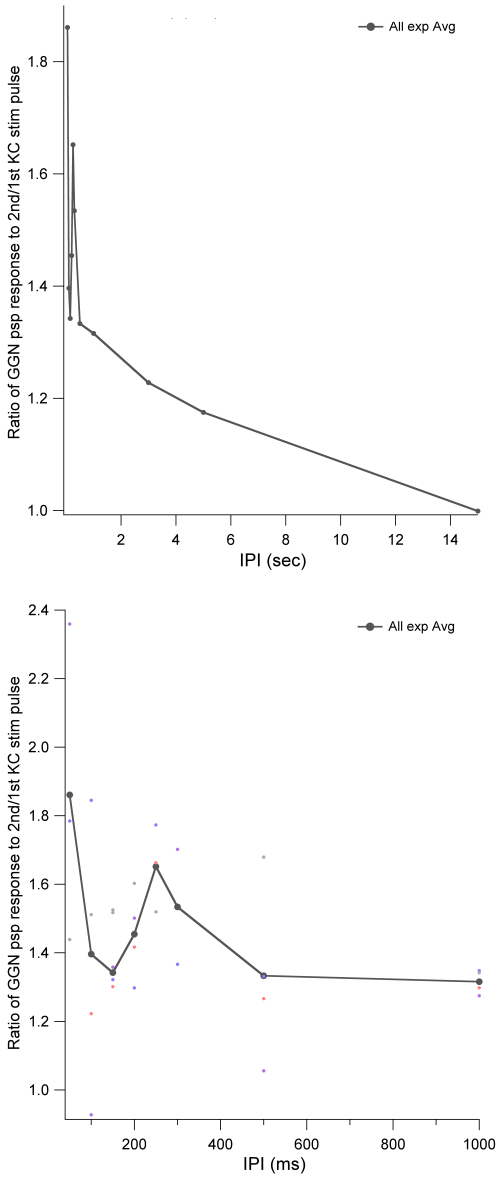


Figure 4.3. Facilitation dependence on IPI. In 4 experiments, we varied the inter pulse interval between two KC stimulation pulses and recorded the change in GGN's membrane potential, ie the ratio of the second pulse over the first pulse. Top panel shows interval for 50ms to15sec. Bottom panel shows average (in black) for the 50ms- 1sec interval, overlaid with all points from all experiments (colored points, same color: points came from same experiment). Note: not all values were tested in all experiments.

4.2.2 Lack of Direct Antennal lobe input in GGN

In Chapter 2 we showed that KCs provide direct input to GGN. During odor stimulation projection neurons (PNs) provide input to KCs, and as such have an indirect effect on GGN. Here we wanted to examine whether PNs also provide direct input into GGN. Anatomically it is a possibility, since their processes overlap in the calyx of the MB and the lateral horn. In addition we have shown in Chapter 2 (Figure 1Dii) that the GGN membrane potential tracks PN output (as assessed by LFP) very well. To address this question we placed a stimulating electrode in the antennal lobe (AL) near PN soma clusters and recorded intracellularly from GGN while stimulating PNs (Perez-Orive et al., 2004). Different stimulation strengths evoked a response in GGN with variable long delays and shapes that were not indicative of a direct monosynaptic connection.This is in contrast to the effect of KC stimulation on GGN, ie reproducible and delay in the order of a few ms. Interestingly, as we increased the PN stimulation strength we observed an increase in inhibition onto GGN. As the PN stimulation is increased, more and more PNs would be recruited, and as result activate more KCs (Perez-Orive et al., 2004).

Presumably the way in which KCs are activated through electrical PN stimulation is more optimal for activating the neuron that inhibits GGN than GGN itself. Given our facilitation results, it would be interesting to examine whether two stimulation pulses in PNs within the KC-GGN facilitation window, could overcome this large inhibitory effect on GGN. At this point, we cannot exclude that PNs -in addition to KCs- provide input to this inhibitory cell. Taken together, we conclude that the postsynaptic response observed in GGN, is indirect and must be due, at least in part, to activation of KCs, which as we have shown in Chapter 2, provide direct input to GGN.

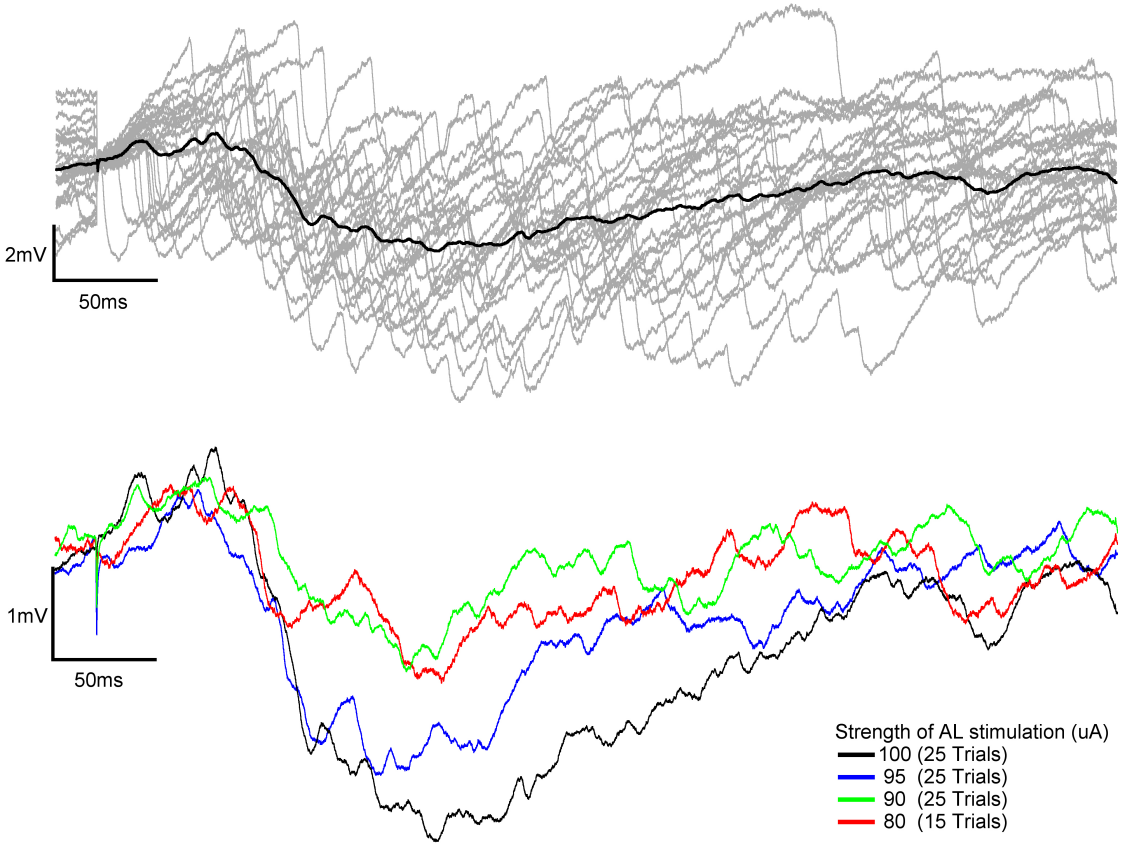


Figure 4.4. GGN Response to AL stimulation. Stimulation of PN somata activates GGN indirectly. Top panel, GGN response to highest stimulation, single trials shown in grey with the average (black) overlaid. Bottom panel shows 25 trial average GGN response to four different stimulation strengths. Note: negative deflection represents stimulation pulse artifact

4.2.3 Assessing KC output through β lobe LFP recordings

β LOBE LFP RESPONSE TO ODOR CONCENTRATIONS

The PN population encodes an increase in concentration by an increase in synchrony (Stopfer et al., 2003). As described in the introduction, this increase can be observed in the LFP recorded in the calyx[3] where PNs send their axons. We have argued that given this increase in synchrony and the fact that KCs act as coincidence

[3]It can also be observed in the KC soma layer

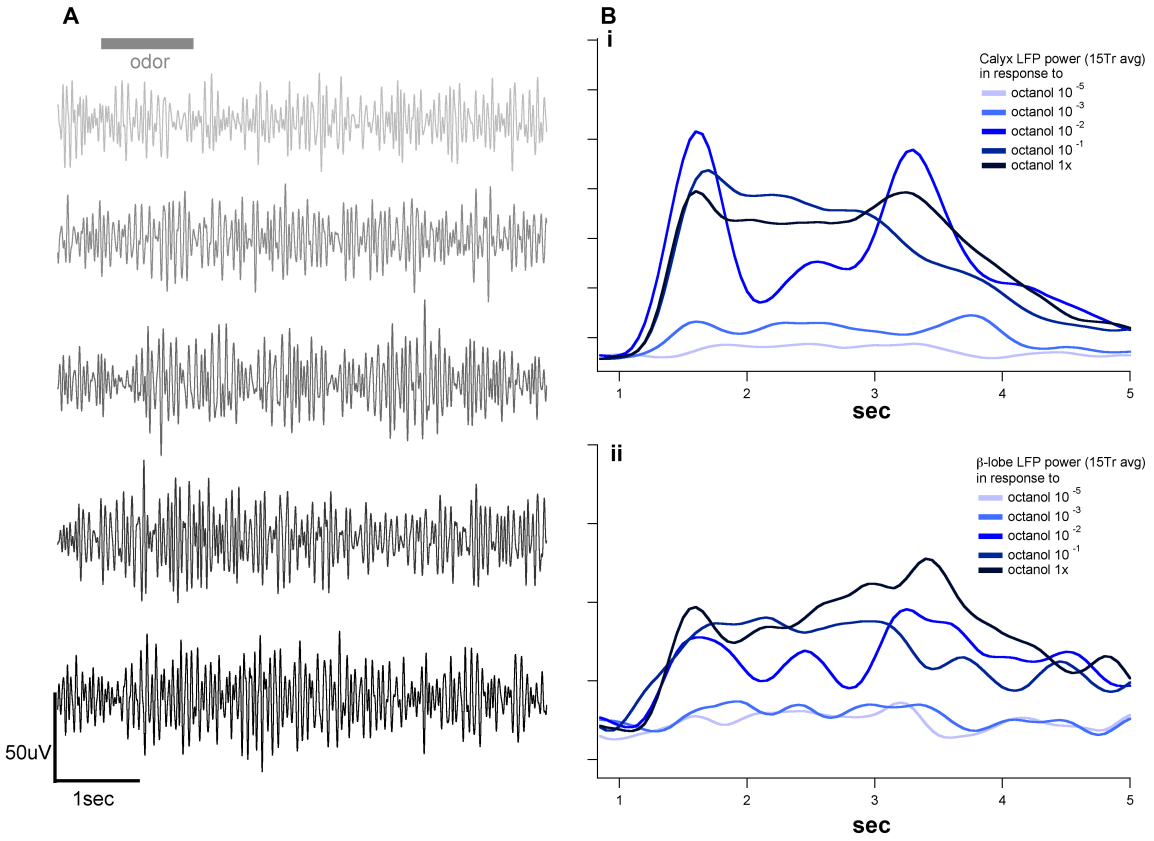


Figure 4.5. β lobe LFP Increases with an Increase in Odor Concentration. **A.** Example β lobe LFP traces (band passed 10-30Hz) to increasing odor concentration (top to bottom: lowest to highest octanol concentration, color coding: lighter to darker concentration increases). **B.** Average LFP power (10−30Hz band) over time recorded in the calyx (Bi) and β lobe (Bii) in response to increasing odor concentration. LFP power was smoothed to facilitate the comparison. Octanol concentrations used: 10^{-5}, 10^{-3}, 10^{-2}, 10^{-1}, 10^{1}.

detectors, there would be a great need for gain control. We have shown further that GGN is in a position to provide this gain control, by counteracting this increase. One interesting question is whether there is an analogous increase in LFP power with increase in odor concentration that is maintained at the KC output, the β lobe, or whether GGN eliminates or attenuates this effect.

To address this issue, we recorded LFP in the β lobe, where KCs make output synapses. Given that LFP is thought to reflect input into an area better than output we hypothesize that such a measure would be a reflection of the total KC activity.

Indeed, such experiments have shown that there still exists an increase in β LFP with an increase in concentration (Figure 4.5). This effect is probably a combination of increase in KC firing and KC synchrony.

EFFECT OF GGN MANIPULATION DURING ODOR ON β LOBE LFP

In addition to the intracellular β lobe recordings shown in Chapter 2, we used the LFP in the β lobe as an alternative way of examining how the KC output is affected during an odor as a result of enhancing GGN beyond the depolarization in response to odor stimulus alone. Consistent with our βLN intracellular data, the power in the β lobe LFP in the 10−30 Hz decreases as a result of this manipulation, confirming GGN's ability to affect KC output (Figure 4.6).

4.2.4 GGN Ablation

Lastly we wondered what would be the network effect of taking GGN altogether out of the circuit. An advantage of having only one GGN per hemisphere is that one can ablate the neuron and observe the effect of the perturbation on the network.

This experiment turned out to be more difficult than we had anticipated. We attempted to kill the cell by injecting it first with carboxyeosin, a dye that upon exposure to light becomes phototoxic. In one such experiment, detailed below, we were successful in ablating GGN, and the results of this manipulation are consistent with GGN's role as described in Chapter 2.

Approach: We injected 5% carboxyeosin in GGN for 45min, using 1Hz negative pulses ranging from 1 to 4nA. Every aspect of this experiment prior to activation of the dye was performed under a red light, outside the dye's excitation spectrum. The LFP in response to odor was recorded in the KC soma layer, simultaneously

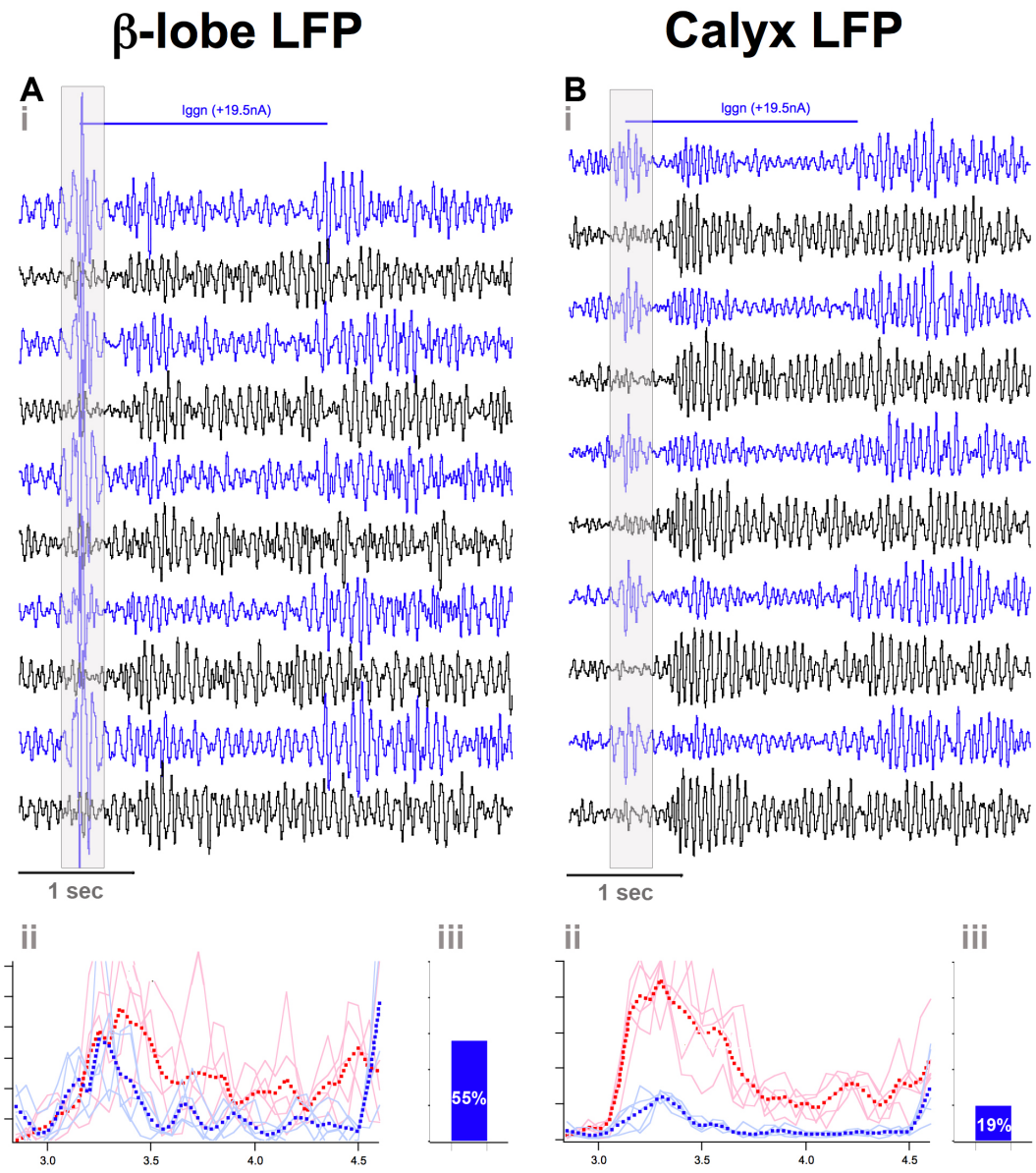
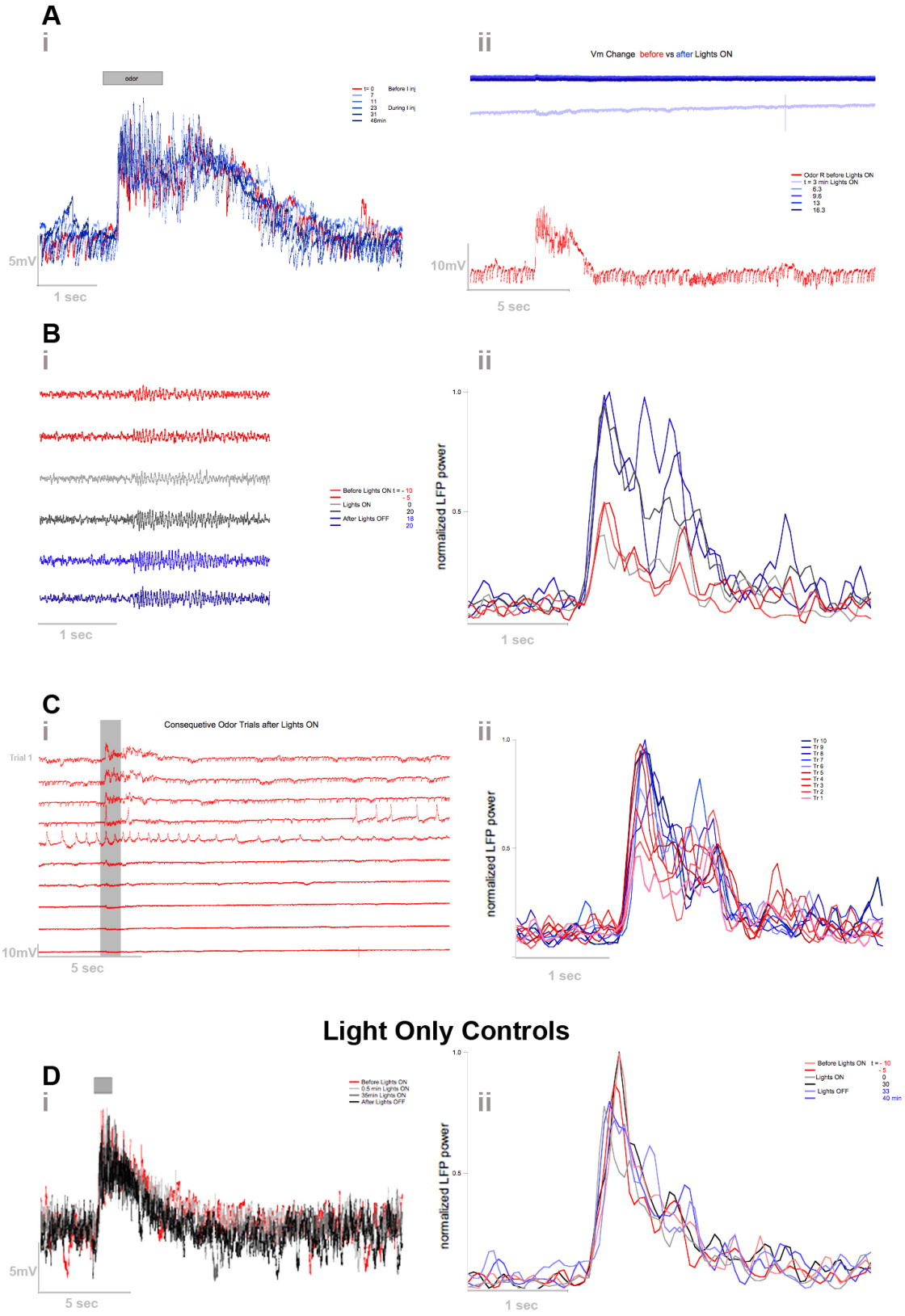


Figure 4.6. Enhancing GGN activity during Odor decreases LFP power in the calyx & β lobe. Effect on β lobe (A) and calyx (B) LFP of injecting current in GGN during odor presentation to enhance its activity. Current injected in GGN (blue trials) during odor is interleaved with odor only trials (black). **A.** Effect on β lobe LFP; (Ai) Example of consecutive single LFP trials (band passed 10−30Hz) and corresponding cumulative power in that range for odor only (control, red) and odor & current injection in GGN. (Aiii). Integral of power over the GGN current injection interval expressed as percent of control. **B.** Same as in (A) except for the LFP recorded in the calyx. GGN current injection: blue bar (2 sec), Odor: horizontal grey bar (1 sec). Light vertical bar: current injection artifact that is picked up by both LFP recording electrodes; This artifact (and an interval of equal size at the end of the pulse) are excluded from any LFP power calculation.

with GGN's intracellular response, before the start of the injection, during and after. As expected, injection of the dye, without light activation did cause any not change the odor response measured in GGN or in the LFP. Upon exposure to the blue light that maximally excites carboxyeosin, GGN's response to odor started to deteriorate. In parallel, as GGN was progressively deteriorating, the power in the simultaneous LFP was increasing. This result demonstrates the direct effect of GGN on KC synaptic currents as assessed by the global LFP measure. Following GGN ablation, the power in the LFP increased by $\sim$ 300%. These results are in strong agreement with the experiments with current injection into GGN during odor stimulation, highlighting the considerable influence GGN exerts over KCs by counteracting PN synchrony and thus maintaining sparse KC activity.

We were particularly interested in repeating these experiments using multiple measures to assess how the subthreshold KC activity and KC output are affected, similarly to the GGN manipulation experiments described in Chapter 2. In addition we were interested in determining how the power in the LFP would change with GGN out of the circuit in response to different odor concentrations. Unfortunately this experiment proved particularly difficult. As its name suggests, this cell is quite large and perhaps the precise location of electrode impalement is critical; GGN is targeted blindly in the locust brain, so it is likely that we target the cell at different

Figure 4.7 ***(on the next page).*** Effect of ablating GGN on LFP power. **A.** Recordings of GGN before (Ai) and after lights ON (Aii). GGN response before, during & after injection of 5% carboxyeosin(CE) confirms there is no dye effect on GGN's membrane potential (Ai) until the blue light is turned on and CE becomes photo-toxic. **B.** Effect on LFP of killing GGN. Single trial LFP traces bandpassed (5-55Hz) are shown in Bi and the corresponding cumulative LFP power in the same band are shown for comparison. **C.** Trial by trial comparison of GGN as it killed (Ci) and the LFP power (in the 5-55Hz band) for the corresponding trial (Cii).**D.** Experiment assessing the effect of light alone on GGN membrane potential (Di) and LFP power (Dii).


A
i
odor
t= 0
7
11
23
31
46min
Before I inj
During I inj
5mV
1 sec
ii
Vm Change before vs after Lights ON
Odor R before Lights ON
t = 3 min Lights ON
6.3
9.6
13
16.3
10mV
5 sec
B
i
Before Lights ON t = - 10
- 5
Lights ON 0
20
After Lights OFF 18
20
1 sec
ii
normalized LFP power
1.0
0.5
1 sec
C
i
Consequetive Odor Trials after Lights ON
Trial 1
10mV
5 sec
ii
normalized LFP power
1.0
0.5
Tr 10
Tr 9
Tr 8
Tr 7
Tr 6
Tr 5
Tr 4
Tr 3
Tr 2
Tr 1
1 sec
Light Only Controls
D
i
Before Lights ON
0.5 min Lights ON
35min Lights ON
After Lights OFF
5mV
5 sec
ii
normalized LFP power
1.0
0.5
Before Lights ON t = - 10
- 5
Lights ON 0
30
Lights OFF 33
40 min
1 sec

locations in different experiments. Perhaps in failed experiments we only managed to sever a branch , which might have been insufficient to eliminate GGN's function. Even though we explored different conditions for enhancing the dye's effect (pH, solvent), we were not able to effectively ablate GGN again. Nevertheless, it was encouraging that the results of the experiment where we did manage to ablate GGN were in complete agreement with the experiments using current injection in GGN during odor stimulation.

In summary, this data strongly suggests that there are no direct connections from PNs to GGN. It speaks to the existence of short term facilitation between KCs and GGN. It also demonstrates that the effect of current injection into GGN during odor stimulations on KC output can also be evaluated on the LFP in the β lobe. And lastly it shows that taking GGN out of the circuit by ablation greatly impacts the LFP power in the calyx, leading to a ~300% increase, which illustrates the prominent influence of GGN.

4.3 Methods

4.3.1 Preparation and stimuli

All results in this study were obtained *in vivo* from locusts (*Schistocerca americana*) that are housed in an established, crowded colony. Young adults of either sex were immobilized in a holder. Both antennae were secured in place with respect to the olfactory delivery system and remained intact for olfactory stimulation. The brain was exposed, desheathed and superfused with locust saline, as previously described (Laurent and Naraghi, 1994).

4.3.2 Odor Delivery

Odors were diluted 10% volume/volume in paraffin oil. Different odor concentration were prepared by serial dilutions, ie 10^{-2} is 1:100 dilution of odor in paraffin oil. Odors were delivered by injection of a controlled volume of odourized air within a constant stream of dessicated air. Total airflow was set t 0.85 L/min and the odor was further diluted by 1/3 in air. Teflon tubing was used at and downstream from the mixing point to prevent odour lingering and cross-contamination.

4.3.3 GGN Intracellular recordings

Sharp electrode recordings from large GGN process along the peduncle were made with borosilicate glass micropipettes (DC resistance, 60MΩ) filled with 3M K acetate. Input resistance was ~15MΩ and the resting membrane potential -51.9 ± 4.9mV. GGN could be recognized by the presence of characteristic IPSPs during baseline and graded response to odor. A series of pilot experiments, in which the recorded cell was stained intracellularly by injection of 6% cobalt hexamine, lucifer yellow or biocytin confirmed it always to have GGN's characteristic anatomy.

4.3.4 Local Field Potential Recordings

Local field potentials in the calyx were always recorded in the mushroom body KC soma layer using Michigan probes. For simultaneous β lobe LFP recordings we used saline-filled patch pipettes (R_{DC}: 2–15 MΩ) or wire tetrodes.

LFP POWER MEASUREMENTS

We measured the average LFP power (in the 10−30 Hz band) as a function of time around an odor pulse. Power was calculated with a scrolling window (width 200

ms, step 50 ms) over all trials (ten per stimulus condition). Injecting current in GGN often caused an artifact so 150 ms on either side of the onset and offset of the current injection were eliminated from each trace (also in control for comparison) prior to power calculation.

4.3.5 KC Electrical stimulation

Twisted-wire tetrodes obtained from FHC (number CE4B75) were modified for monopolar stimulation, with the casing serving as the anode. The tips of the tetrodes were splayed such that the distance between the exposed tips was approximately equal to 60% of the diameter of the mushroom body calyx. The exposed end of the stimulating electrode was embedded among Kenyon cell somata. The tetrodes were electroplated with gold solution to reduce the impedance to between 200 and 350 kΩ at 1 kHz. Stimulating currents (5–140 μA, 0.1 ms) were generated by an STG1000 Multichannel System.

4.3.6 AL Electrical stimulation

Electrical stimulation of PN somata & processes was performed in the AL, using 25 μm tungsten wire bipolar electrodes and a WPI A360 stimulus isolator (World Precision Instruments, Sarasota, FL) at 300 μsec pulses; stimulus amplitudes shown here were 80-100 μA.

Chapter 5

Conclusions & Future directions

In the introduction I discussed the need for gain control in the mushroom body (MB), based on an argument that encompasses the connectivity between projection neurons (PNs) and kenyon cells (KCs), the threshold of KC firing and the number of neurons involved (Jortner et al., 2007). In this thesis I have characterized a paired giant GABAergic neuron with extensive arborizations in the MB, which provides this necessary gain control. We have demonstrated the existence of a feedback loop between KCs and GGN through which GGN provides inhibition to each KC that is scaled by the total KC activity.

We have also identified an additional level of control built into the system, which consists of mutual inhibition between GGN and another neuron. This neuron also appears to be driven by KC input and provides tonic inhibition onto GGN during baseline, suggesting that it is important to minimize basal neurotransmitter release from GGN.

By manipulating GGN activity during odor, we observed a prominent effect on KC subthreshold activity, both as measured at the single-cell level as well in the local field potential (LFP) recorded in the MB. This LFP is considered to be an aggregate of synaptic currents into KCs and as such provided a way for examining

GGN's effect on subthreshold activity across the KC population. One way GGN could potentially exert this effect is through changes in the KCs' input resistance that could effectively shunt incoming excitatory input.

In order to determine the effect of this manipulation on KC output, we recorded from a previously characterized population of MB extrinsic neurons that receive KC input in the β lobe (β-LNs, (Cassenaer and Laurent, 2007)). We selected these cells for several reasons; first there are ~ 30 such cells, each receiving input from a several thousands of KCs, therefore allowing us to monitor the effect of GGN on a large number of KCs. Secondly, since these cells transmit the activity of the KCs to areas downstream of the MB, they provide a functional read-out for the effect of our manipulation. Depolarizing GGN beyond its endogenous level during odor stimulation demonstrated the ability of this cell to shut down almost entirely the output from KCs. Conversely, hyperpolarizing GGN during odor stimulation led to an increase in β-LN firing. These manipulations were powerful as well as immediately reversible.

Given that there is only one GGN per hemisphere, we were interested in ablating the cell and recording potential effects on KC activity. While, this task proved to be quite difficult, in one successful experiment we observed that following cell ablation there was a significant increase in the power of the LFP recorded in the MB, consistent with the GGN current-injection results. Furthermore, as the effect of the ablation progressed, the odor-evoked increase in the LFP power correlated well with the decay of the membrane potential in GGN on a trial-by-trial basis.

Lastly, we have characterized a *Drosophila* neuron that appears to share many of the locust GGN properties, strengthening our hypothesis that GGN could be a general solution for gain control in the MB. A single clone of the GH146 line

(generated by Greg Jefferis, using the MARCM technique) labeled this cell, and revealed a morphology similar to the locust GGN, with extensive arborizations throughout the MB calyx and lobes. By means of intracellular recording, we have confirmed that it is a non spiking neuron, and that it has a graded response to odors of a similar time course as its locust counterpart. Furthermore, it responds to increasing odor concentration with an increase in membrane depolarization, consistent with providing additional inhibition at higher concentrations, much like the locust GGN.

In summary, we have shown that GGN controls the gain of the MB by normalizing the output of the KC population, thereby reducing the dependence on the overall strength of the input. We have shown this in detail in the locust, and we have demonstrated that an analogous neuron with very similar properties exists in the fly.

5.1 Future directions:

This study has provided some answers, but it has also raised questions and possibilities for exploring and extending some of our findings to further address the role of gain control, as well some critical issues related to its implementation. We think that some aspects of these results might be generalizable to other systems, given the widespread instantiation of gain control at many different levels and nervous systems. (Salinas and Sejnowski, 2001; Reynolds and Chelazzi, 2004; Winkowski and Knudsen, 2006; Olsen and Wilson, 2008; Winkowski and Knudsen, 2008; Saalmann and Kastner, 2009; Robinson and McAlpine, 2009; Laurent, 1993)

5.1.1 Locust

One question relates to the dynamic range at which GGN sets the output of the KC population. Based on preliminary recordings, we hypothesized in Chapter 2 that GGN could be important for setting the KC output within a range over which STDP can operate. STDP was recently found to govern the synapses between KCs and β-LNs, and to maintain the β-LN firing at a particular phase of the LFP (Cassenaer and Laurent, 2007). In our GGN manipulation experiments, we observed that when GGN was inhibited during the odor, the firing of β-LNs increased, such that they often fired multiple times within a given cycle. We argued that by placing β-LNs in such a regime, STDP will not be able to recover the typical phase of the β-LNs. For these experiments we used negative current injection in GGN; this manipulation was often successful, but in some cases seemed quite dependent on the location of GGN impalement. Based on our recent discovery of the cell that inhibits GGN, we have a much more effective way of (synaptically) controlling GGN activity. By activating this cell during the odor we could more profoundly inhibit GGN and examine the downstream effects. This manipulation would be reversible and, as such, superior to GGN ablation (which was also technically very difficult).

These experiments would also be useful in examining the multiple layers of control that exist in the system. We would like to know, for example, how the activity of the inhibitory cell is generated. This cell appears to keep the extent of GGN depolarization in check, particularly during baseline, which in turn affects the responsiveness of KCs. Sparseness and gain control, as well as modulation of gain control itself, can be found in many other systems and in many brain areas. The insect nervous system presents a unique opportunity to study these issues, given that these functionalities are localized in a very small number of

individual neurons that can be precisely controlled and used to perturb the system in a reversible manner.

An additional characteristic of the GGN response that would be compelling to study in further detail, is the presence of short term facilitation of the synapses made by KCs onto GGN. The role of this facilitation is as yet uncertain; we think it might effectively implement KC lateral inhibition. As we have shown in Chapter 4, a second KC spike within a certain time-window (which is a very typical occurrence) results in a facilitated response in GGN. As such, KCs that are activated for the second time can enhance their effect on GGN and raise the threshold for other KCs that did not yet fire at that cycle. Additionally, such an effect could be necessary to ensure that GGN is activated during the odor, but not during baseline. As mentioned previously, both GGN and the other inhibitory neuron receive input from KCs. The other neuron has a high basal firing rate, which could be explained if it connects to all 50,000 KCs. During odor stimulation the balance shifts, with GGN being mostly activated and the inhibitory neuron mostly inhibited. Given that the number of KCs activated during the odor is small (~ 10% cumulatively over the duration of the response), perhaps GGN relies on this facilitation to boost its activation state over the inhibitory neuron, thus enabling it to provide the necessary inhibition onto KCs.

GGN is important for maintaining KC sparseness, but there are other mechanisms that contribute significantly as well, such as intrinsic KC dendritic properties and feedforward inhibition. Ideally we would like to understand the system to such an extent that by modeling many of its components, we can predict the responses of the system at various levels. Such efforts are already underway (Shen *et al* in preparation) and this understanding appears within reach.

5.1.2 *Drosophila*

Drosophila GGN provides an opportunity to assess the role of gain control in the MB behaviorally. The necessary experiments for providing such a link are detailed in Chapter 3. A preliminary step to conclusively demonstrate that GGN acts to inhibit KCs in the fruit fly, as it does in the locust, would be to combine intracellular recordings in GGN with imaging of KC activity. The goal would be to assess the effect of manipulating GGN on KC activity. The next step would be to use a specific line that selectively labels only GGN, and to target a channel that would allow us to remotely manipulate GGN activity. Using this line we can assess how reversibly tuning KC population activity would influence the animals' performance in an appetitive or aversive conditioning paradigm. This would differ from previous studies where subsets of KCs have been silenced during such behavioral tasks, since here all KCs would be affected. Furthermore, our results in the locust, together with the proposed imaging experiments provide a framework through which these results can be interpreted. In the locust, the KC to β-LN synapse is known to be governed by STDP (Cassenaer and Laurent, 2007) and recent results show that a neuromodulator implicated in associative conditioning can change the shape of the STDP curve, providing a description of synaptic changes that would underly learning (Cassenaer *et al* in preparation). Given the profound effect on the β-LN population of manipulating GGN during odor stimulation, we would expect the results of the behavioral experiments to be informative.

References

Adrian E (1942) Olfactory reactions in the brain of a hedgehog. *J. Physiol. (Lond.)* 100:459–73.

Adrian ED (1950) The electrical activity of the olfactory bulb. *E.E.G. Clin. Neurophysiol.* 2:377–88.

Andersen RA, Zipser D (1988) The role of the posterior parietal cortex in coordinate transformations for visual-motor integration. *Can J Physiol Pharmacol* 66:488–501.

Barlow HB (1972) Single units and sensation: a neuron doctrine for perceptual psychology? *Perception* 1:371–94.

Benzer S (1967) Behavioral mutants of drosophila isolated by countercurrent distribution. *Proc Natl Acad Sci USA* 58:1112–1119.

Bhandawat V, Olsen SR, Gouwens NW, Schlief ML, Wilson RI (2007) Sensory processing in the drosophila antennal lobe increases reliability and separability of ensemble odor representations. *Nat Neurosci* 10:1474–82.

Bitterman M MRFASS (1983) Classical conditioning of proboscis extension in honeybees (apis mellifera). *J. Comp. Physiol.* 97:107–19.

Boyden ES, Zhang F, Bamberg E, Nagel G, Deisseroth K (2005) Millisecond-timescale, genetically targeted optical control of neural activity. *Nat Neurosci* 8:1263–8.

Brand AH, Perrimon N (1993) Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* 118:401–15.

Broome BM, Jayaraman V, Laurent G (2006) Encoding and decoding of overlapping odor sequences. *Neuron* 51:467–482.

Buck LB (1996) Information coding in the vertebrate olfactory system. *Annu. Rev. Neurosci.* 19.

Buck L, Axel R (1991) A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell* 65:175–87.

Burrows M, Boeckh J, Esslen J (1982) Physiological and morphological properties of interneurons in the deutocerebrum of male cockroaches with responses to female pheromones. *J. Comp. Physiol.* A 145:447–57.

Campbell RAA, Turner GC (2010) The mushroom body. *Curr Biol* 20:R11–2.

Carandini M, Heeger DJ (1994) Summation and division by neurons in primate visual cortex. *Science* 264:1333–6.

Cassenaer S, Laurent G (2007) Hebbian stdp in mushroom bodies facilitates the synchronous flow of olfactory information in locusts. *Nature* 448:709–713.

Chance FS, Abbott LF, Reyes AD (2002) Gain modulation from background synaptic input. *Neuron* 35:773–82.

Claridge-Chang A, Roorda RD, Vrontou E, Sjulson L, Li H, Hirsh J, Miesenböck G (2009) Writing memories with light-addressable reinforcement circuitry. *Cell* 139:405–15.

Crittenden JR, Skoulakis EM, Han KA, Kalderon D, Davis RL (1998) Tripartite mushroom body architecture revealed by antigenic markers. *Learn Mem* 5:38–51.

DasGupta S, Waddell S (2008) Learned odor discrimination in drosophila without combinatorial odor maps in the antennal lobe. *Curr Biol* 18:1668–74.

Davis RL (1993) Mushroom bodies and drosophila learning. *Neuron* 11:1–14.

Davis RL (2004) Olfactory learning. *Neuron* 44:31–48.

Davis RL (2005) Olfactory memory formation in drosophila: From molecular to systems neuroscience. *Annual Review of Neuroscience* 28:275–302.

de Belle J, Heisenberg M (1994) Associative odor learning in Drosophila abolished by chemical ablation of mushroom bodies. *Science* 263:692–695.

DeWeese MR, Wehr M, Zador AM (2003) Binary spiking in auditory cortex. *J Neurosci* 23:7940–9.

Dravnieks A (1982) Odor quality: semantically generated multidimensional profiles are stable. *Science* 218:799–801.

Dubnau J, Grady L, Kitamoto T, Tully T (2001) Disruption of neurotransmission in drosophila mushroom body blocks retrieval but not acquisition of memory. *Nature* 411:476–80.

Fahrbach SE (2006) Structure of the mushroom bodies of the insect brain. *Annu Rev Entomol* 51:209–32.

Field DJ (1994) What is the goal of sensory coding. *Neural Comput* 6:559–601.

Friedrich RW, Laurent G (2001) Dynamic optimization of odor representations by slow temporal patterning of mitral cell activity. *Science* 291:889–94.

Gronenberg W (2001) Subdivisions of hymenopteran mushroom body calyces by their afferent supply. *J Comp Neurol* 435:474–89.

Hahnloser RH, Kozhevnikov AA, Fee MS (2002) An ultra-sparse code underlies the generation of neural sequences in a songbird. *Nature* 419:65–70.

Hallem EA, Carlson JR (2004) The odor coding system of drosophila. *Trends Genet* 20:453–9.

Hallem EA, Carlson JR (2006) Coding of odors by a receptor repertoire. *Cell* 125:143–60.

Hamilton KA, Kauer JS (1989) Patterns of intracellular potentials in salamander mitral,tufted cells in response to odor stimulation. *J Neurophysiol* 62:602–25.

Han X, Boyden ES (2007) Multiple-color optical activation, silencing, and desynchronization of neural activity, with single-spike temporal resolution. *PLoS ONE* 2:e299.

Heisenberg M, Borst A, Wagner S, Byers D (1985) Drosophila mushroom body mutants are deficient in olfactory learning. *J. Neurogenet.* 2:1–30.

Holt GR, Koch C (1997) Shunting inhibition does not have a divisive effect on firing rates. *Neural computation* 9:1001–13.

Hotta Y, Benzer S (1970) Genetic dissection of the drosophila nervous system by means of mosaics. *Proc Natl Acad Sci USA* 67:1156–63.

Jayaraman V, Laurent G (2007) Evaluating a genetically encoded optical sensor of neural activity using electrophysiology in intact adult fruit flies. *Front Neural Circuits* 1:3.

Jefferis GS, Marin EC, Stocker RF, Luo L (2001) Target neuron prespecification in the olfactory map of drosophila. *Nature* 414:204–8.

Jefferis GSXE, Marin EC, Watts RJ, Luo L (2002) Development of neuronal connectivity in drosophila antennal lobes and mushroom bodies. *Curr Opin Neurobiol* 12:80–6.

Joiner WJ, Crocker A, White BH, Sehgal A (2006) Sleep in drosophila is regulated by adult mushroom bodies. *Nature* 441:757–60.

Jortner R, Farivar SS, Laurent G (2007) A simple connectivity scheme for sparse coding in an olfactory system. *J Neurosci* 27:1659–1669.

Kanerva P (1988) *Sparse Distributed Memory* MIT Press, Cambridge, MA.

Kasuya J, Ishimoto H, Kitamoto T (2009) Neuronal mechanisms of learning and memory revealed by spatial and temporal suppression of neurotransmission using shibire, a temperature-sensitive dynamin mutant gene in drosophila melanogaster. *Front Mol Neurosci* 2:11.

Kauer J MD (1974) Responses of olfactory bulb neurones to odour stimulation of small nasal areas in the salamander. *J. Physiol. (Lond.)* 243:717–37.

Kaupp UB (2010) Olfactory signalling in vertebrates and insects: differences and commonalities. *Nat Rev Neurosci* 11:188–200.

Keene AC, Krashes MJ, Leung B, Bernard JA, Waddell S (2006) Drosophila dorsal paired medial neurons provide a general mechanism for memory consolidation. *Curr Biol* 16:1524–30.

Keene AC, Waddell S (2005) Drosophila memory: dopamine signals punishment? *Curr Biol* 15:R932–4.

Keller A, Vosshall LB (2004) Human olfactory psychophysics. *Curr Biol* 14:R875–8.

Kitamoto T (2001) Conditional modification of behavior in drosophila by targeted expression of a temperature-sensitive shibire allele in defined neurons. *J Neurobiol* 47:81–92.

Kitamoto T (2002) Conditional disruption of synaptic transmission induces male-male courtship behavior in drosophila. *Proc Natl Acad Sci U S A* 99:13232–7.

Krashes MJ, Keene AC, Leung B, Armstrong JD, Waddell S (2007) Sequential use of mushroom body neuron subsets during drosophila odor memory processing. *Neuron* 53:103–115.

Laurent G (1993) A dendritic gain control mechanism in axonless neurons of the locust, schistocerca americana. *J Physiol Lond* 470:45–54.

Laurent G (1999) A systems perspective on early olfactory coding. *Science* 286:723–8.

Laurent G (2000) What does understanding mean? *Nat Neurosci* 3:1211.

Laurent G (2002) Olfactory network dynamics and the coding of multidimensional signals. *Nat Rev Neurosci* 3:884–95.

Laurent G, Davidowitz H (1994) Encoding of olfactory information with oscillating neural assemblies. *Science* 265:1872–5.

Laurent G, Naraghi M (1994) Odorant-induced oscillations in the mushroom bodies of the locust. *J Neurosci* 14:2993–3004.

Laurent G, Stopfer M, Friedrich RW, Rabinovich MI, Volkovskii A, Abarbanel HD (2001) Odor encoding as an active, dynamical process: experiments, computation, and theory. *Annu Rev Neurosci* 24:263–97.

Laurent G, Wehr M, Davidowitz H (1996) Temporal representations of odors in an olfactory network. *J Neurosci* 16:3837–47.

Lee T, Lee A, Luo L (1999) Development of the drosophila mushroom bodies: sequential generation of three distinct types of neurons from a neuroblast. *Development* 126:4065–76.

Leitch B, Laurent G (1996) Gabaergic synapses in the antennal lobe and mushroom body of the locust olfactory system. *J Comp Neurol* 372:487–514.

Lennie P (2003) The cost of cortical computation. *Curr Biol* 13:493–7.

Lima SQ, Miesenbock G (2005) Remote control of behavior through genetically targeted photostimulation of neurons. *Cell* 121:141–52.

Linster C, Smith B (1999) Generalization between binary odor mixtures and their components in the rat. *Physiol. Behav.* 66:701–7.

Liu X, Davis RL (2009) The gabaergic anterior paired lateral neuron suppresses and is suppressed by olfactory learning. *Nat Neurosci* 12:53–9.

MacLeod K, Laurent G (1996) Distinct mechanisms for synchronization and temporal patterning of odor-encoding neural assemblies. *Science* 274:976–9.

Marr D (1969) A theory of cerebellar cortex. *J Physiol* 202:437–70.

Masse NY, Turner GC, Jefferis GSXE (2009) Olfactory information processing in drosophila. *Curr Biol* 19:R700–13.

Mazor O, Laurent G (2005) Transient dynamics versus fixed points in odor representations by locust antennal lobe projection neurons. *Neuron* 48:661–73.

McGuire SE, Le PT, Davis RL (2001) The role of drosophila mushroom body signaling in olfactory memory. *Science* 293:1330–3.

Miesenbock G (2004) Genetic methods for illuminating the function of neural circuits. *Curr Opin Neurobiol* 14:395–402.

Miesenbock G, De Angelis DA, Rothman JE (1998) Visualizing secretion and synaptic transmission with ph-sensitive green fluorescent proteins. *Nature* 394:192–5.

Mitchell SJ, Silver RA (2003) Shunting inhibition modulates neuronal gain during synaptic excitation. *Neuron* 38:433–45.

Mombaerts P (1999) Seven-transmembrane proteins as odorant and chemosensory receptors. *Science* 286:707–11.

Mombaerts P, Wang F, Dulac C, Chao SK, Nemes A, Mendelsohn M, Edmondson J, Axel R (1996) Visualizing an olfactory sensory map. *Cell* 87:675–86.

Murthy M, Fiete I, Laurent G (2008) Testing odor response stereotypy in the drosophila mushroom body. *Neuron* 59:1009–23.

Nakai J, Ohkura M, Imoto K (2001) A high signal-to-noise ca(2+) probe composed of a single green fluorescent protein. *Nat Biotechnol* 19:137–41.

Ng M, Roorda RD, Lima SQ, Zemelman BV, Morcillo P, Miesenbock G (2002) Transmission of olfactory information between three populations of neurons in the antennal lobe of the fly. *Neuron* 36:463–74.

Olsen SR, Bhandawat V, Wilson RI (2007) Excitatory interactions between olfactory processing channels in the drosophila antennal lobe. *Neuron* 54:89–103.

Olsen SR, Wilson RI (2008) Lateral presynaptic inhibition mediates gain control in an olfactory circuit. *Nature* 452:956–60.

Olshausen BA, Field DJ (2004) Sparse coding of sensory inputs. *Curr Opin Neurobiol* 14:481–7.

Perez-Orive J, Bazhenov M, Laurent G (2004) Intrinsic and circuit properties favor coincidence detection for decoding oscillatory input. *J Neurosci* 24:6037–47.

Perez-Orive J, Mazor O, Turner GC, Cassenaer S, Wilson RI, Laurent G (2002) Oscillations and sparsening of odor representations in the mushroom body. *Science* 297:359–65.

Pitman JL, DasGupta S, Krashes MJ, Leung B, Perrat PN, Waddell S (2009) There are many ways to train a fly. *Fly (Austin)* 3:3–9.

Poo C, Isaacson JS (2009) Odor representations in olfactory cortex: "sparse" coding, global inhibition, and oscillations. *Neuron* 62:850–61.

Prescott SA, Koninck YD (2003) Gain control of firing rate by shunting inhibition: roles of synaptic noise and dendritic saturation. *Proc Natl Acad Sci USA* 100:2076–81.

Pulver SR, Pashkovski SL, Hornstein NJ, Garrity PA, Griffith LC (2009) Temporal dynamics of neuronal activation by channelrhodopsin-2 and trpa1 determine behavioral output in drosophila larvae. *J Neurophysiol* 101:3075–88.

Quinn WG, Harris WA, Benzer S (1974) Conditioned behavior in drosophila melanogaster. *Proc Natl Acad Sci U S A* 71:708–12.

Raman B, Joseph J, Tang J, Stopfer M (2010) Temporally diverse firing patterns in olfactory receptor neurons underlie spatiotemporal neural codes for odors. *J Neurosci* 30:1994–2006.

Reynolds JH, Chelazzi L (2004) Attentional modulation of visual processing. *Annu Rev Neurosci* 27:611–47.

Robinson BL, McAlpine D (2009) Gain control mechanisms in the auditory pathway. *Curr Opin Neurobiol* 19:402–7.

Saalmann YB, Kastner S (2009) Gain control in the visual thalamus during perception and cognition. *Curr Opin Neurobiol* 19:408–14.

Salinas E, Sejnowski TJ (2001) Gain modulation in the central nervous system: where behavior, neurophysiology, and computation meet. *Neuroscientist* 7:430–40.

Sato K, Pellegrino M, Nakagawa T, Nakagawa T, Vosshall LB, Touhara K (2008) Insect olfactory receptors are heteromeric ligand-gated ion channels. *Nature* 452:1002–6.

Schroll C, Riemensperger T, Bucher D, Ehmer J, Völler T, Erbguth K, Gerber B, Hendel T, Nagel G, Buchner E, Fiala A (2006) Light-induced activation of distinct modulatory neurons triggers appetitive or aversive learning in drosophila larvae. *Curr Biol* 16:1741–7.

Shang Y, Claridge-Chang A, Sjulson L, Pypaert M, Miesenbock G (2007) Excitatory local circuits and their implications for olfactory processing in the fly antennal lobe. *Cell* 128:601–612.

Shepherd G (1993) Principles of specificity and redundancy underlying the organization of the olfactory system. *Microsc. Res. Tech.* 24:106–12.

Shepherd G (1994) Discrimination of molecular signals by the olfactory receptor neuron. *Neuron* 13:771–90.

Smith B MR (1989) The use of electromyogram recordings to quantify odorant discrimination in the honeybee, apis mellifera. *J. Insect Physiol.q* 35:369–75.

Stocker RF, Heimbeck G, Gendre N, de Belle JS (1997) Neuroblast ablation in drosophila p[gal4] lines reveals origins of olfactory interneurons. *J Neurobiol* 32:443–56.

Stopfer M, Jayaraman V, Laurent G (2003) Intensity versus identity coding in an olfactory system. *Neuron* 39:991–1004.

Strausfeld NJ, Hansen L, Li Y, Gomez RS, Ito K (1998) Evolution, discovery, and interpretations of arthropod mushroom bodies. *Learn Mem* 5:11–37.

Su CY, Menuz K, Carlson JR (2009) Olfactory perception: receptors, cells, and circuits. *Cell* 139:45–59.

Suh GS, Wong AM, Hergarden AC, Wang JW, Simon AF, Benzer S, Axel R, Anderson DJ (2004) A single population of olfactory sensory neurons mediates an innate avoidance behaviour in drosophila. *Nature* .

Sutherland C, Doiron B, Longtin A (2009) Feedback-induced gain control in stochastic spiking networks. *Biological cybernetics* 100:475–89.

Tempel BL, Bonini N, Dawson DR, Quinn WG (1983) Reward learning in normal and mutant drosophila. *Proc Natl Acad Sci USA* 80:1482–6.

Tian L, Hires SA, Mao T, Huber D, Chiappe ME, Chalasani SH, Petreanu L, Akerboom J, McKinney SA, Schreiter ER, Bargmann CI, Jayaraman V, Svoboda K, Looger LL (2009) Imaging neural activity in worms, flies and mice with improved gcamp calcium indicators. *Nat Methods* 6:875–81.

Tully T, Quinn WG (1985) Classical conditioning and retention in normal and mutant drosophila melanogaster. *J Comp Physiol [A]* 157:263–77.

Turner GC, Bazhenov M, Laurent G (2008) Olfactory representations by drosophila mushroom body neurons. *J Neurophysiol* 99:734–46.

Vinje WE, Gallant JL (2000) Sparse coding and decorrelation in primary visual cortex during natural vision. *Science* 287:1273–6.

Vosshall LB, Amrein H, Morozov PS, Rzhetsky A, Axel R (1999) A spatial map of olfactory receptor expression in the drosophila antenna. *Cell* 96:725–36.

Vosshall LB, Wong AM, Axel R (2000) An olfactory sensory map in the fly brain. *Cell* 102:147–59.

Wehr M, Laurent G (1996) Odour encoding by temporal sequences of firing in oscillating neural assemblies. *Nature* 384:162–6.

Wehr M, Laurent G (1999) Relationship between afferent and central temporal patterns in the locust olfactory system. *J Neurosci* 19:381–90.

Wilson RI, Laurent G (2005) Role of gabaergic inhibition in shaping odor-evoked spatiotemporal patterns in the drosophila antennal lobe. *J Neurosci* 25:9069–79.

Wilson RI, Turner GC, Laurent G (2004) Transformation of olfactory representations in the drosophila antennal lobe. *Science* 303:366–70.

Wilson RI, Mainen ZF (2006) Early events in olfactory processing. *Annual Review of Neuroscience* 29:163–201.

Winkowski DE, Knudsen EI (2006) Top-down gain control of the auditory space map by gaze control circuitry in the barn owl. *Nature* 439:336–9.

Winkowski DE, Knudsen EI (2008) Distinct mechanisms for top-down control of neural gain and sensitivity in the owl optic tectum. *Neuron* 60:698–708.

Withers GS, Fahrbach SE, Robinson GE (1993) Selective neuroanatomical plasticity and division of labour in the honeybee. *Nature* 364:238–40.

Yasuyama K, Kitamoto T, Salvaterra PM (1996) Differential regulation of choline acetyltransferase expression in adult drosophila melanogaster brain. *J Neurobiol* 30:205–18.

Yasuyama K, Meinertzhagen IA, Schurmann FW (2002) Synaptic organization of the mushroom body calyx in drosophila melanogaster. *J Comp Neurol* 445:211–26.

Yasuyama K, Meinertzhagen IA, Schurmann FW (2003) Synaptic connections of cholinergic antennal lobe relay neurons innervating the lateral horn neuropile in the brain of drosophila melanogaster. *Journal of Comparative Neurology* 466:299–315.

Yu D, Keene AC, Srivatsan A, Waddell S, Davis RL (2005) Drosophila dpm neurons form a delayed and branch-specific memory trace after olfactory classical conditioning. *Cell* 123:945–57.

Zhang F, Wang LP, Brauner M, Liewald JF, Kay K, Watzke N, Wood PG, Bamberg E, Nagel G, Gottschalk A, Deisseroth K (2007a) Multimodal fast optical interrogation of neural circuitry. *Nature* 446:633–9.

Zhang K, Guo JZ, Peng Y, Xi W, Guo A (2007b) Dopamine-mushroom body circuit regulates saliency-based decision-making in drosophila. *Science* 316:1901–4.