# Synopsis

**Title**  
Neural correlates of Olfactory learning in *Drosophila melanogaster*

**Name of the candidate**  
Tuhin Subhra Chakraborty

**Subject**  
Life Science

**Degree**  
Doctor of Philosophy

**Name of the Guide**  
Prof. Obaid Siddiqi

**Institution**  
National Center for Biological Sciences  
Tata Institute of Fundamental Research  
GKV K Campus, Bellary Road  
Bangalore: 560065
Title: Neural correlates of olfactory learning in *Drosophila melanogaster*

Olfaction and olfactory conditioning in fruit fly, *Drosophila melanogaster* have been investigated in many laboratories. Our understandings of genetic and molecular biology of olfaction and olfactory learning have greatly increased in recent years (Davis 2005, Vosshall 2007, Waddell S, 2001). Many studies concerning the effect of odor exposure on behavior have focused on parasitoids. The first suggestions came from Thorpe and Jones (1937). They showed that female ichneumonid parasitoids, *Venturia carnescens*, could be conditioned to odors of wax moth larvae, *Meliphora grisella*, a host to which they were not normally attracted, when reared on this unusual host. The behavior of parasitoid can be influenced by prior exposure to odors. Subsequent studies by Thorpe (1939) showed that when *Drosophila* larvae were cultured in a peppermint scented medium; the flies emerging from these cultures acquired an induced preference for peppermint. He called the phenomenon pre-imaginal conditioning or larval imaginal conditioning. Thorpe’s experiments were repeated by others but his interpretation was questioned (Manning 1967). Manning concluded that Thorpe effect was not due to conditioning but was a result of habituation. In a later reinvestigation of the phenomenon, Barron and Corbet (1999) found no evidence to support the carryover of larval conditioning to the adult flies, but observed that exposure to menthol changes behavior.

Experiments carried out in our laboratory addressed to the effect of certain esters and alcohols on the imago after eclosion (Panchal P et.al., 1997). This phenomenon has been called imaginal conditioning in order to distinguish it from pre-imaginal conditioning or the Thorpe effect. Balaji Krishnan (1996) using binary trap showed that the imago exhibits a biphasic response to odors. At low concentrations, the flies are attracted to odorants such as ethyl acetate, iso-amyl acetate and n-butanol. Beyond an optimal concentration, attraction gives way to repulsion. Punita Panchal and Firdos Khan (1999) found that the response curves for a set of odorants, mainly acetate esters and alcohols are dramatically changed by early exposure to odorants. Flies kept on an odor less sucrose-agar medium exhibit minimal attraction to chemicals (Hisham M, Unpublished). However, when exposed to specific odorants, they develop a strong
preference for the odors which they have experienced and an aversion to odors that they had not encountered.

Imaginal conditioning studied in the lab has certain features common with associative learning. Nixon Abraham (2001) and Divya Sekhar (Unpublished) showed that when sucrose is given before and after odor exposure, attraction is significantly reduced. However, if flies are kept in an environment devoid of odor and sucrose (reward), the olfactory memory does not decay at all suggesting a relatively stable part of memory that last for several days (Abraham 2001). The neurophysiological effects of imaginal conditioning on the imago after eclosion have been investigated in the present study.

Chapter 3 describes the effect of imaginal conditioning on the sensitivity of chemoreceptors. In *Drosophila* imago, about 300 chemoreceptors (sensillum) cover the antennae and maxillary palp (Venkatesh and Singh, 1984). The olfactory sensilla fall into different morphological types known as basiconica, trichoidea and coeloconic (Venkatesh and Singh, 1984, Shanbagh SR 1999). Whereas basiconica sensilla are found on both the antennae and maxillary palp, trichoid and coeloconic sensilla are located exclusively on the third antennal segment and serve distinct chemosensory function (Siddiqi O, 1983, de Bruyne, 2001, Yao CA et.al., 2005, Carlson JR, 2010). On the basis of their response spectra, de Bruyne et.al., (2001) have classified sensilla basiconica into seven types. Similar study was performed by Anil Gupta (1995) from a group of eighteen sensilla present above the sacculus on the third antennal surface.

2. In the present study, an extensive electrophysiological characterization of three sensilla basiconica was made in order to examine the effect of imaginal conditioning on chemoreceptors. The three chemoreceptors studied respond to esters, ketones and alcohols. Type I sensillum has four neurons whose spike amplitude falls into four classes. The larger amplitude spike referred as type IA, respond to ethyl butyrate. The other neuron in this sensillum are IB, IC and ID, respond to ethyl acetate, CO₂ (de Bruyne et.al., 2000) and methyl salicylate respectively (Gupta A, 1995). Spikes from type II and III sensillum resolved into two populations based on their spike amplitude.
3. Conditioning with ethyl acetate and iso-amyl acetate greatly enhances the sensitivity of the neurons present in type I and type II sensillum respectively. The induced increase in firing frequency obtained in single unit recording is pronounced at lower concentrations. Although the increase in electrophysiological response continues up to 72 hours of post eclosion, most of the enhancement occurs in the first 30 hours of conditioning. Odor induced response to three esters and one alcohol were characterized in type II sensillum. Similar to the case of ethyl acetate conditioning where most enhancement of odor sensitivity was to EA, ethyl butyrate (EB) and iso-amyl iso valarate (IV) conditioning led to particular enhancement to EB and IV respectively.

4. A set of olfactory mutants were characterized by comparing conditioned with unconditioned responses to a set of chemicals in our behavioral assay (Rashid B et.al., NCBS Report, 2005). These mutants have been named ‘Icon’ (for imaginal conditioning). On the basis of their phenotypes, icon mutants can be grouped into different classes. Class I mutants show diminished induced response (icon-), class II mutants exhibit enhanced response (iconen) and class III show increased uninduced or constitutive response. Jawaid Ahsan (2009) has performed molecular characterization of these mutants and the P-element insert point was localized by either plasmid rescue or inverse PCR or both. Electrophysiological recordings were made with these mutants for various odorants and found that in two out of eight mutants, the odor induced response at the receptor level reduced.

5. Flies were cultured under three different conditions. The first group was raised on odor less medium during the larval and imaginal stages; the second group was raised on cornmeal medium during the larval stage and an odor less medium during the imaginal stage; the third group was raised on an otherwise odor less medium containing $10^{-4}$ dilution of EA. Strong difference exist between the response of these classes of flies with first group responding the least to the chosen set of chemicals: ethyl acetate, iso-amyl-iso-valarate, ethyl valarate, ethyl butyrate and iso-amyl butyrate and the last group responding the most. When both larva and imago were grown on cornmeal, the
enhancement in sensitivity depends upon the composition of the medium. Difference disappears when flies were conditioned.

In order to study whether increased sensitivity at the receptor level lowers sensory threshold in the axon terminals of the olfactory sensory neurons (OSNs) and its cognate projection neurons (PNs), we employed in vivo two photon Ca\textsuperscript{2+} imaging. Chapter 4 shows that flies conditioned to EA exhibits hypersensitivity to ethyl acetate in Or42b OSN terminals at lower concentrations. Similar observations were made with IAA conditioned flies. It has been found that EA induced flies also show enhanced odor response in DM1 projection neurons. No increment in sensitivity observed when OSN output is blocked. Volume changes at the glomerular level were found significantly different when flies were conditioned with EA. The early exposure to n-hexanol increases the volume of Or22a OSN housed in type III sensillum.

Chapter 5 describes the effect of starvation on the sensitivity of receptor neurons. Starvation elicits increased attraction to food odorants such as ethyl acetate, 2-3 butanedione, 1-hexanol and ethyl propionate respectively. Increment in firing response is more at the lower concentrations barely significant at higher concentrations. Both starvation and imaginal conditioning increases the firing frequency of IIA neuron but not to the same extent. Effect of induction is more pronounced when conditioning is followed by starvation. \textit{Drosophila} neuropeptide F (dNPF) has been implicated in motivational control of appetitive behavior in adult flies (Shen P 2001). It has been found that blocking dNPF mimics satiation. OSNs do not exhibit any increases in firing frequency to food odorants in flies bearing dNPF-Gal4 and UAS-TNT-G transgenes suggesting that dNPF acts as a motivational switch that control the appetite.

Chapter 6 provides an information theoretic analysis of ensemble spike responses to certain esters: ethyl acetate, ethyl butyrate and iso-amyl iso valarate and alcohols in type IIA neuron. Jensen-Shanon divergence (DJS), a metric from information theory was employed to assess quantitatively the difference in olfactory acuity of flies grown in three different culture media, a) an odor free synthetic medium, b) synthetic medium
supplemented with a single odorant and c) cornmeal medium, a rich source of many odorants. Statistical analysis shows that flies exposed to an odorant have increased sensitivity to the odors without much improvement in odor discrimination. Deprivation of odor leads to loss of both sensitivity and odor discrimination. Interestingly, fly cultured on cornmeal medium exhibits varied sensitivity but develop greater ability to distinguish odors.

In summary, the research work presented in this thesis demonstrates that imaginal conditioning is associated with increased electrophysiological responses of single neurons housed in large sensilla basiconica. OSNs primarily serve to drive the activity of the downstream neurons in the olfactory pathway. In vivo two photon functional imaging experiments establish the link between increased sensitivity at the level of OSNs and hypersensitivity in the axon terminals of OSNs and its cognate projection neurons, leading to an increase in attraction.

References


Unpublished Results

