Imaginal olfactory conditioning
A project proposal for the ___ Plan.

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My group works on olfactory perception in *Drosophila*. Our ~~research to ask~~ is centered ~~around~~ around a set of genes which regulate olfaction. The aim is to gain an understanding of the brain mechanisms that ~~between~~ between perception of chemicals. The assumption underlying our approach (shared by others in the field) was that olfactory responses of *Drosophila* are stereotyped and ~~to~~ rigidly determined at birth. In recent years we have found that the above assumption is wrong. Our experiments show that attraction and aversion to odours are learnt responses. Flies grown in an 'odour-free' environment do not exhibit olfactory responses. If, after eclosion the imago (adult fly) is exposed to particular odorants, these odorants become

attractants. We have designated this phenomenon imaginal conditioning, in order to distinguish it from a related effect larval conditioning, discovered by W G Thorpe. There are reasons to believe that imaginal conditioning is widespread in insects as well as other phyla. Experiments in our group show, that, phenomenologically speaking, imaginal conditioning is akin to associative learning and involves ~~short~~ ~~long~~ term and long term memory.

Aims and experimental approach:

Our objective is to determine the neural correlates of olfactory conditioning, including its localization in specific regions of the flys brain. As a result of the past work of several groups at TIFR (mine, Prof V. Rodrigues, Prof R.N. Singh and Prof G. Hazan) and elsewhere in Europe and USA there exists an unusually detailed knowledge of olfactory behaviour, neuroanatomy, ~~and~~ electrophysiology and molecular biology in Drosophila. This will be a great advantage in the study of olfactory conditioning.

Our experimental approach will continue to be based on neurogenetics. We have a collection of over 25 olfactory genes. The molecular products of some of these have been identified. The Drosophila genome has now been completely sequenced. The entire set of olf genes can be analyzed rapidly and their protein products can be identified.

The new olf mutants in our collection have been generated by a UAS-Gal4 transposon that contains β-galactosidase and GFP as reporters and the gene for tetanus toxin which can be used to block synapses. We can attempt to map the brain regions where olf genes express with a high degree of precision. I expect that, at least some of the olf genes affect imaginal conditioning. One of the aims of this project will be to establish operational criteria which will distinguish conditioning pathway genes from genes that impair sensory response.

Physiology of Drosophila CNS

Over these years my group has carried out detailed studies on electrophysiological response in the antenna. We now have a nearly complete description of the response spectra of different antennal sensilla.

We now wish to concentrate on the electrophysiology of the central nervous system. The possible approaches to CNS physiology in Drosophila are single unit electrode recordings, optical recordings and functional mapping, using methods such as ^{3}H-deoxyglucose, earlier done by Prof. V. Rodrigues. A major obstacle in this direction is the small size of Drosophila brain. I propose to overcome this difficulty by using X-ray shadowgraphy while recording. The technical feasibility of this approach will be investigated in collaboration with Dr. Shivkumar (at NCBS) and Prof. M.A. Vishwamitra (at I.Sc). If exploratory results are encouraging we will take up the construction of an X-ray projection microscope

A secon line of effort in sensory

physiology will be directed towards in vitro studies in neuronal cultures. Dr. Shivshankar is interested in developing single-molecule interaction techniques for studying biochemical events including olfactory transduction. My laboratory is equipped for cell culture and patch clamp physiology. I hope to develop this project in collaboration with Dr Shiv Shankar and others. The overall aim of our research will be understanding the biophysical and cellular basis of olfactory transduction.

Financial Requirement.

1. Behaviour Genetics and molecular biology will be continuation of ongoing work. Principal requirements are consumables & small equipment and miscellaneous items — 30 Lakhs
2. Computer automation of behavioural experiments. We propose to automate several of our behavioural paradigms (track test, larval test and flight response) using automated counting and videography

Main items will be PCs, CCD cameras, Software for tracking 20 Lakhs

3. X-ray shadowgraphing for electrophysiology:
The project will be taken up only if feasibility studies are successful.
Main expensive items are X-ray source and X-ray CCD camera 25 Lakhs

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Total 75 Lakhs

Personnel:

Most of the project will be carried out with students. Post Docs and other visiting ~~Scientists~~ scientists I would however like to have 2 scientific assistants on a relatively long term ~~Contract~~ contract for electronic and engineering assistance and for Drosophila stock keeping.