Sanjay P. Sane PI Report

Work plan for AOARD May 2008-April 2010
PROGRESS REPORT AND EXTENDED WORKPLAN

May 2008- July 2008: Basic Setup

1. Implementation of High-Speed Videography **(Completed)**
2. Implementation of Motion Analysis tools and acquisition software **(Completed)**
3. Design and Initiation of Wind Tunnel-Treadmill Construction **(Completed)**
4. Set up of Soldier fly colony **(Completed)**

August 2008-October 2008: Setup and Preliminary Experiments

5. Development of a flight tube for studying Take-off and Landing behavior **(Completed)**
6. Behavioral Experiments on Soldier flies (*Hermetia Illucens*) to elicit controlled take-off and landing in free flight **(Pilot experiments completed. Follow- up experiments in progress)**
7. Behavioral experiments on insects in wind tunnel-treadmill **(Treadmill and wind tunnel constructed. Behavioral experiments have already begun on tethered Moths and freely-flying bees in the wind tunnel)**
8. Begin construction of panels to provide controlled visual stimuli to insects. **(Not started)**

October 2008-January 2009: Experiments and Analysis

9. Experiments within combined vision-mechanosensory environments.**(First set of experiments completed. See report on Musca landing)**
10. Development of data analysis software **(Completed)**
11. Analysis of preliminary behavioral data **(Completed)**
12. Neuroanatomical and pharmacological studies **(In progress)**

January 2009-May 2009: Follow-up experiments

1. Follow up experiments to test hypothesis on Drosophila food finding and landing (**Pending**)
2. Continuation of pilot behavioral experiments on soldier flies for landing and take-off (**First set of experiments completed. See report**).
3. Completion of Wind tunnel-Treadmill installation (**completed and calibrated**).

June 2009- August 2009: Optical Grid setup and Wind tunnel experiments

4. Design and Construction of optical grid to localize insects in 2D. This grid will allow us to conditionally move a virtual object depending on the position of the approaching insect. The apparatus will be designed to also fit the wind

Report Documentation Page

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14. ABSTRACT

The goal of this research is to develop three flight assays to offer a comparative test of various behaviors under combinations of visual and mechanisensory stimuli. These assays will be used to quantify behavioral latencies of flying insects.

15. SUBJECT TERMS
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tunnel.(**First version of the apparatus was tested, an improved version 2 in progress**)

5. Wind tunnel-treadmill experiments on fruit flies (**Wind tunnel experiments on bees and moths well underway**).

September 2009-January 2009: Apiary set up and work on bees.

6. Set up an apiary and trained bees to fly upwind within the wind tunnel.
7. Pilot behavioral experiments with role of antenna in bee flight.

Overview: Research in my laboratory focuses on diverse aspects of insect flight ranging from aerodynamics and sensory neurobiology to behavior. We investigate the neural basis of selected flight behaviors, such as landing, take-off and sharp turns which occur in time scales of a few wing strokes. Because such behaviors typically involve simpler sets of neural connections, we hope that their study will allow us to address more complex behaviors which are composed of these smaller modular behaviors (e.g. a territorial chase between houseflies is composed of a take-off followed by many sharp turns). In pursuing this goal, we have decided to not restrict ourselves to any one "model" system, but instead chosen diverse insect systems in which these questions are best addressed. Such a broad approach is necessary to establish the generality of our questions

Over the past year, we have investigated the following questions:

1) Antennal positioning behavior in the moth, *Daphnis neerii*.
2) Location of odor sources in the fruit fly, *Drosophila melanogaster*.
3) Wing-haltere coordination in the soldier fly, *Hermetia illucens*.
4) Landing behavior in the housefly, *Musca domestica*.

We have also recently established an apiary and are routinely using bees in many experiments. The following sections describe ongoing work in each of these areas.

1. Antennal positioning behavior in the moth, *Daphnis neerii* (Anand Krishnan, Subashini Sudarshan, Sunil Prabhakar): Two sets of antennal mechano-sensors are located in the scapal and pedicellar segments of the antenna. One set, the Bohm's bristles, are organized orthogonally as fields of bristles on the surface of the scape and pedicel. Another set, the Johnston's organs are circumferentially embedded within the pedicel-flagellar joint. Unlike the scape and the pedicel which move actively due to their segmental muscles, the flagellum has no muscles and only moves passively. The Johnston's organs sense the passive motion between the pedicel-flagellar joint.

Recently, we showed that antennal mechanosensors play a crucial role in flight control. When the Johnston's organs were unloaded by cutting the flagellum above the pedicel-flagellar joint, insects lost control of their flight trajectory. When the Johnston's organs were reloaded by gluing the cut flagellum to its stub, insects regained control of their flight trajectory. Intracellular recordings from individual scolopidial units of the Johnston's organs and physical calculations of mechanical forces acting on the antenna during flight indicated that Johnston's organs encode Coriolis strains at the base of antennae, similar to halteres in Diptera. We are investigating various aspects of these

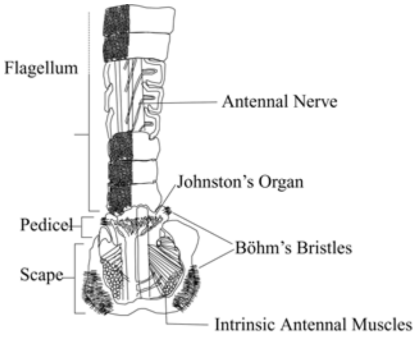

Fig 1: Antennal Anatomy

phenomena in greater detail to understand the nature of the stabilizing mechanosensory input provided by the antenna to the flight motor.

At rest, moths retract their antennae underneath their wings. However, during flight, their antennae move forward and are held at constant angles irrespective of the oncoming airflow which would normally drag the antennae back. This antennal positioning behavior is found not just in all Lepidoptera, but also insects of other orders such as Hymenoptera. Which antennal mechanosensory structures mediate the antennal positioning response? How does the antennal positioning response factor into the larger question of antennal mechanosensory input during flight? We have addressed this question using diverse methods including behavior, neuroanatomy and neurophysiology.

On the behavioral front, we developed a method to perturb the antennal position in tethered flapping moths and measure its recovery. From these experiments, we showed that the normal antenna rapidly and stereotypically recovers its position. However, if the Bohm's bristles are ablated, the antenna fails to properly position during flight or recover from perturbations. Moreover, this response appears to be primarily mediated by the scapal bristles, with the pedicellar bristles (which are far fewer in number) exerting a subtler effect. We have also showed that the Johnston's organs may not be involved in antennal positioning. These experiments provided clear evidence that it is the Bohm's bristles that primarily mediate antennal positioning responses in moths.

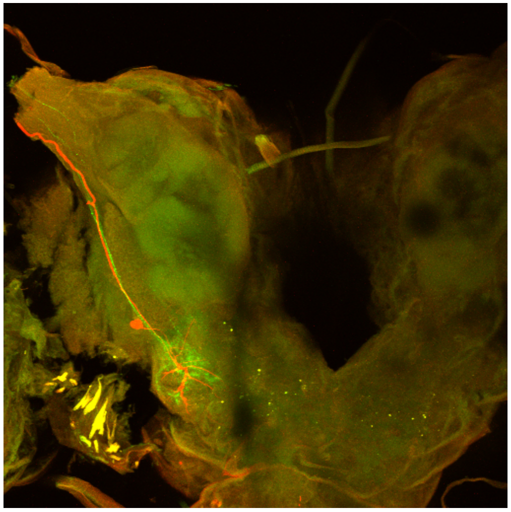

Fig 2. Double dye fills of the Bohm's bristle arbors (green) and antennal motor neurons (red). The two overlap in the deutocerebral region of the brain called Antennal Motor and Mechanosensory Center (AMMC)

Where do the mechanosensory bristle neurons communicate information about changes in antennal positioning to the antennal motor system? To visualize the underlying neural connectivity, we performed neuro-anatomical investigations involving double-dye fills of the sensory and motor ends of the antennal system. These experiments reveal heavy overlap of the Bohm's bristle arbors with the dendritic branches of the antennal motor neurons suggesting direct connectivity between these two systems. To test this hypothesis, we performed electrophysiological recordings of the antennal muscles while stimulating their bristles. Preliminary results from these experiments confirm that the Bohm's bristles indeed activate the antennal muscles over short time scales. Based on these investigations, we can conclude that the antennal positioning reaction is mediated by the Bohm's bristle system *via* a simple negative feedback loop which reports any changes in the set point position of the antenna, and activates the antennal motor neurons to mitigate these changes.

These results are particularly exciting because the antennal positioning response appears to be a classic reflex arc every component of which is experimentally accessible. Its connectivity is simple and behavior is easily measurable, this preparation holds great promise for future single sensor level investigations (e.g. encoding and adaptation properties of the bristle neurons) as well as systems level questions regarding the development and evolution of antennal positioning. Our preliminary study of the Bohm's bristles across a diversity of insect orders shows that the bristles and their underlying connectivity are conserved features in all Neoptera. Do these bristles serve similar purpose in all these insects? How do these structures adapt to the diversity of antennal positioning behaviors? Finally, why do flying insects of these orders keep their antennal angles constant? These are just a few of the many questions we can begin to address with the techniques developed during this study.

2. Location of odor sources in the fruit fly, *Drosophila melanogaster* (Nitesh Saxena, Rana Kundu): We have assembled a system to quantify the 3D flapping movement of insect wings and body with high temporal resolution using high-speed videography. Unlike traditional approaches which were limited by the need for very bright illumination thereby hindering natural behavior, our setup is sensitive to infrared (IR) light which is invisible to fruit flies. Thus, by conducting behavioral experiments under IR illumination, we can ensure naturalistic insect behavior.

Using this system, we have begun to study how fruit flies pinpoint the location of an odor source (banana mash placed within a black pole, a strong visual landmark against a visually barren landscape). In the presence of a single, visible odor source, flies punctuate their rapid approach with a hovering phase some distance above the object. Following the hovering phase, the fly rapidly descends on the odor source. In presence of two or more objects, only one of which contains an odor source, they initiate a search response, hovering over each object until it finds the source of odor (Fig 3). When a fly is presented with an invisible odor source placed some distance away from a single black object, its trajectory

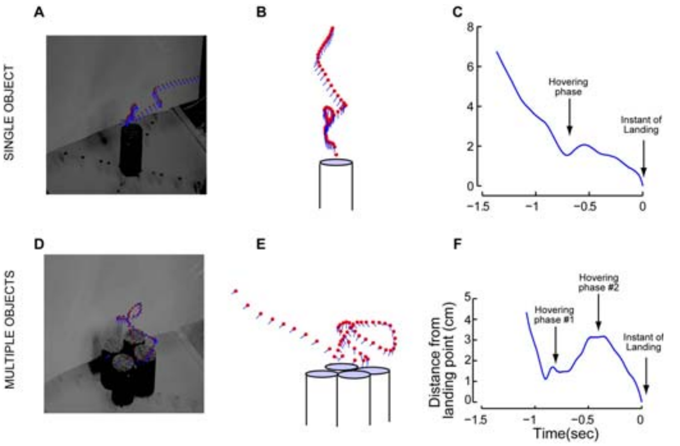

Figure 3: Odor tracking in freely flying *Drosophila melanogaster*. (A-C): Fly tracking a single visual and odor object. **(D-F):** Fly tracking a single odor object but multiple visual objects. **(A, D):** Frames from one of the high-speed cameras. The fly's trajectory is depicted as a ball and stick model in which the red ball represents the fly's head and the blue stick represents the fly's body. The time difference between any two sticks is 25 ms. The fly was filmed at 4000 frames per second. **(B, E):** 3D trajectories reconstructed from the high-speed videography. **(C, F):** Approach curves for odor tracking flies: Blue line depicts the distance of the fly from its ultimate landing spot as a function of time. The fly shows a single hovering phase in the approach curve when tracking a single object **(C)** and multiple hovering phases when tracking multiple objects **(F).**

is confined to the volume between the odor source and the visual object, suggesting that it uses both cues for navigation. However, after hovering in this volume for a while, it eventually lands on the visual object rather than the odor source. Taken together, our results suggest that the fly consolidates information from both visual and olfactory inputs before making landing decisions.

If the fly depends on a synthesis of olfactory and visual inputs to identify odor targets, then specific rules of navigational decision-making may be required when the fly attempts to identify an odor among a clutter of objects. We hope to uncover these rules, using an experimental approach that relies on presenting conditions of varying relative magnitudes of the sensory stimuli and tracking the flight trajectories of flies as they try to find the odor source within this complex sensory environment. We will provide near and far field visual cues of varying contrast and varying odor concentrations. We will also increase visual complexity by providing several visual objects, only one that coincides with the odor source, and we will assay the ability of flies to correctly (performance accuracy) and rapidly (speed of performance) identify the odor source. At a later stage, we will add mechanosensory information on top of olfactory and visual cues. How is the navigational-decision making process modified under such conditions? How do navigational algorithms adapt to challenges of increasing complexity? The answers to these questions will shed light on the navigational algorithms utilized by adult *Drosophila* during flight towards olfactory targets.

3. Wing-haltere coordination in the soldier fly, *Hermetia illucens* (Tanvi Deora): One of the key sensory inputs for flight stability in Diptera comes from the haltere system. Halteres acts as vibrational gyroscopes and inform the flies' nervous systems about the angular velocity of their body during flight. Using this system in conjunction with vision, flies are able to perform complex aerial maneuvers. During flight, halteres move precisely anti-phase to the wing motion. Indeed, this tight anti-phase synchronization of the wing and haltere movement appears to be crucial for stable flight. We have recently begun investigating the neural basis of the wing-haltere coordination. We would like to perturb the halteres or wings mid-flight and ask how their kinematics recovers from such perturbations? Are insects unable to control their flight during this recovery time? These are some the questions we are setting up to experimentally address in near future.

4. Landing behavior in the housefly, *Musca domestica* (Sathish Kumar, Rana Kundu, Navish Wadhwa): To understand how flies integrate inputs from multiple sensory modalities during landing, we have devised a behavioral assay to study flies as they land on visual objects that we provide them. This assay relies on the fact that houseflies are attracted to strong contrast visual cues when flying in an otherwise visually barren environment and we can film the flies to study their wing motion in great detail. Using this system, we are addressing basic questions about sensory-motor integration, in addition to the flight mechanics of aerial turns. We are focusing on how flies perform a pitch maneuver. How do they control the aerodynamic torques around their body with alterations in their wing kinematics? The experimental system that we have developed allows us to quantify three-dimensional kinematics (Fig 4). A quasi-steady aerodynamic model I had developed as a graduate student will calculate forces and torques resulting from altered kinematics to address how the fly changes its wing motion to generate pitch maneuvers. We have also successfully developed assays to look at take-off and sharp turns along similar lines.

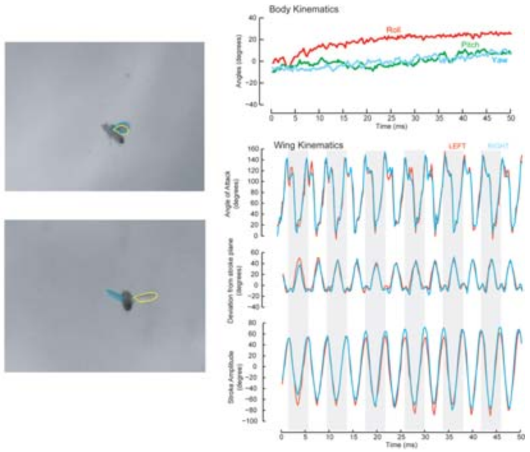

Figure 4: Extracting wing and body kinematics from freely flying *Drosophila melanogaster*. (A) Two frames, each showing the silhouette of the adult fly from different angles. Virtual wire frame models enable an exact reconstruction of the wing position at every time point and the head and tail can be digitized separately from the spatially calibrated video films. **(B)** This enables us to reconstruct the insect's body orientations in the form of pitch (green), roll (red) and yaw (blue) angles and,**(C)** each of three Eulerian angles that characterize the 3D kinematics of both the left (red) and the right (blue) wings.

Major developments that were not previously planned:

1. We decided to begin research on bees with the view that their trainability would allow us to achieve some of the more difficult behavioral assays. This has proved to be a good decision. The National Center for Biological Sciences kindly agreed to give us space to build an insectary, one part of which now houses an apiary which was set up in August-September 2009. This will allow us to take many of our experiments towards somewhat controlled field conditions.

We have been able to train the bees to find their way from the apiary, through a the lab window and a small hole on the wind tunnel, to a feeder placed at the upstream end of a wind tunnel. In the process, we are able to get them to fly upwind while keeping track of their antennal angles and flight trajectory with two high-speed video cameras. We have already begun generating very interesting data on how they position their antennae during free flight. We have also repeated several experiments that I had previously conducted on moths to establish antennal involvement in flight control. It appears from these pilot data, that bees very much resemble the moths in this regard. Because we can train them to perform various maneuvers such as sharp turns, upside down landing etc., we are now seriously looking at bees as a model system for these studies. This begins a very exciting phase of research using bees as a model system.

2. We undertook experiments to train the moth, *Daphnis neerii* in various contexts with a view of a long-term plan to use this insect as a model system. These trainings have also been very successful, making *D. neerii* an exciting system of study. Many future experiments will use trained moths to elicit a wide variety of behaviors such as hover feeding, flower tracking, odor tracking etc.