

Final Report

Sensory coordination of insect flight

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14. ABSTRACT This report contains the investigation results to 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.					
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Objectives of Report

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 nerii*.
- 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.

Status of effort:

May 2008- July 2008: Basic Setup

1. Implementation of High-Speed Videography
2. Implementation of Motion Analysis tools and acquisition software
3. Design and Initiation of Wind Tunnel-Treadmill Construction

August 2008-October 2008: Setup and Preliminary Experiments

4. Development of a flight tube for studying Take-off and Landing behavior
5. Behavioral Experiments on take-off and landing in free flight
6. Behavioral experiments on insects in wind tunnel.
7. Begin construction of panels to provide controlled visual stimuli to insects.

October 2008-January 2009: Experiments and Analysis

8. Experiments within combined vision-mechanosensory environments.
9. Development of data analysis software
10. Analysis of preliminary behavioral data
11. Neuroanatomical and pharmacological studies

January 2009-May 2009: Follow-up experiments

12. Follow up experiments on insect food finding and landing
13. Continuation of pilot experiments on landing and take-off
14. Completion of Wind tunnel-Treadmill installation

June 2009- August 2009:

15. 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 tunnel.
16. Wind tunnel experiments on fruit flies

September 2009-January 2010: Apiary set up and studies of bee flight.

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

January 2010 – May 2010:

20. Construction of outdoor insectary for moth field experiments
21. Moth field studies initiated
22. Musca studies completed.

May 2010- August 2010:

23. Moth antennal positioning studies completed
24. Follow up experiments to test hypothesis on Drosophila food finding and landing in a wind tunnel
25. Optical grid Version 2 expected to come end of October. Currently we are interfacing Version 1 with MATLAB psychophysics tool box to enable conditional visual display.

Personnel not salaried from AOARD funds:

Dr. Sanjay P. Sane (PI)

Dr. Rajesh Sivasankaran (Post-doctoral fellow, joined 2010)

Anand Krishnan (Integrated PhD: Joined 2008)

Nitesh Saxena (Integrated PhD: Joined 2008)

Tanvi Deora (Integrated PhD: Joined 2009)

Taruni Roy (Integrated PhD: Joined 2009)

Sunil Prabhakar (MSc by Research: Joined 2009)

Subashini Sudarshan (2008-2010)

Harshada Sant (joined 2010)

M. Kemparaju (Insect Breeding)

Research Assistants salaried from AOARD funds:

Amit Singh (2008- date)

Aravin Chakravathy (2009- date)

Nihav Dhawale (joined 2010)

Navish Wadhwa (2008-2010)

Publications in 2009-2010:

Sane, S.P., Srygley, R.B. and Dudley, R. (2010). Antennal regulation of migratory flight in the neotropical moth *Urania fulgens*. *Biology Letters*, 6, 406–409.

Sane S.P.* and McHenry M.J. (2009) The biomechanics of sensory organs. *Integrative and Comparative Biology*, 49(6):i8-i23.

Zhao, L., Huang, Q., Deng, X. and Sane, S.P. (2010). Aerodynamic effects of flexibility in flapping wings . *Journal of The Royal Society Interface*, 7, 485-497.

Interactions

Title: Behavioral and Biomechanical insights into Insect Flight Behavior

Invited Speaker, **Wright State University, Dayton, Ohio, USA** (26May 2010)

Title 1: How do insects fly?

Title 2: The neuroethology of insect flight.

Invited speaker, **DST-INSPIRE, Hongirana Public school**, Sagar, Shimoga (Mar 23, 2010)

Title: Dissecting the mechanisms of insect flight behavior

Invited speaker, **Jawaharlal Nehru Center for Advanced Scientific Research** (Mar 23, 2010)

Title: The neuroethology of insect flight.

Invited speaker, **Jawaharlal Nehru Planetarium** (Feb 13 2010)

Title: How do insects fly?

Invited speaker, **TIFR Center for Applicable Mathematics**, (Feb 2 2010)

Title: Insect flight

Invited speaker, **Gandhi Krishi Vigyan Kendra (GKVK)** , India (18 Dec 2010)

Title: On flies and flows: a multidisciplinary perspective on insect flight

Invited Speaker, **National Aerospace Laboratories**, India (Nov, 2009)

Title: Aerodynamics of flapping flight

Invited Speaker, **Symposium: What can we learn from insects?**

Indian Academy of Sciences, India (Nov 2009)

Title: How insects fly: A multi-disciplinary perspective

Invited Speaker, **Department of Aerospace Engineering, Indian Institute of Technology, Kanpur**. (August 2009)

Title: Behavioral and Biomechanical insights into insect flight

Invited Speaker, **Molecular Biophysics Unit, Indian Institute of Science, India** (July 2009)

Title: On flies and flows: behavioral and biomechanical insights into insect flight

Keynote Speaker, **International Symposium on Nature-Inspired Technology 2009-
International Symposium on Intelligent Unmanned Systems 2009**, Jeju, Republic of Korea, June 17-19, 2009

Title: Biomechanical and behavioral insights into insect flight

Invited Speaker, **International Workshop on Nocturnal Pollination**, March 24-27, 2009

Indian Institute of Science, India

Title: Biomechanical and behavioral insights into insect flight

Plenary Speaker, **Functional Biology: Comparative Aspects**, March 19-21, 2009

Department of Zoology, University of Lucknow, Lucknow

Title: The tale of two mechanosensors: antennal control of insect flight

Invited Speaker and Co-organizer, **Sensory Biomechanics Symposium, Society of Integrative and Comparative Biology, Boston, USA** (3-7 Jan 2009)

Inventions: None

Honors/Awards: Ramanujan Fellowship, Department of Science and Technology, India (2010-2015)

Archival Documentation: Description of work

Antennal positioning behavior in the moth, *Daphnis nerii*: (Anand Krishnan, Subashini Sudarshan, Sunil Prabhakar)

Description of the antennal morphology: The base of the antenna contains two sets of mechanosensory structures are located in the scapal and pedicellar segments of the antenna (Fig 1 A-C). One set, called the *Böhm's bristles*, are organized as fields of hair-like sensilla on the surface of scape and pedicel. In Lepidoptera, the scapal and pedicellar fields are organized roughly orthogonally to each other, whereas in Hymenoptera, they are more uniformly arranged around the circumference of the scapal segment (Fig 1 D, E). Another set, the *Johnston's organs* are made of several individual stretch receptor units called scolopidia which are circumferentially arranged around the pedicel-flagellum joint and embedded just under the pedicellar cuticle in both moths and bees. The head capsule-scape joint is moved by a set of *extrinsic muscles* which join the scape to the head capsule, whereas the scape-pedicel joint is moved by a set of *intrinsic muscles* which join the scape to the pedicel (Fig 1 B). Unlike the scape and pedicel which move actively due to their segmental muscles, the flagellum has no muscles and its motion is passive. This passive deformation of the pedicel-flagellar joint is sensed by the individual scolopidial units of the Johnston's organs. The basic morphology of this mechanosensory system is quite conserved among most Neoptera insects, including bees which also contain Böhm's bristles and Johnston's organs in the same relative positions as the moths (Fig 1 D, E).

Antennal Positioning Behavior: In diverse insects including moths, bees, butterflies, beetles etc., the first step towards flight initiation is the stereotypic positioning of the antennae. In moths which tuck their antennae underneath their wings when resting, preparation for take-off begins with a forward positioning of their antennae and control of this position during flight. Although there is great diversity of the external antennal morphology of these diverse insects, the arrangement of the mechanosensory and motor apparatus at the antennal base is mostly conserved. Thus, antennal positioning response in any one system is likely to be informative in addressing this behavior across a wide range of insect taxa. Moreover, antennal positioning behavior may be important to ensure that the mechanosensory signal from the Johnston's organs, which is critical for flight

control, is relatively free of noise from unwanted antennal movements arising from turbulent upwind conditions during flight.

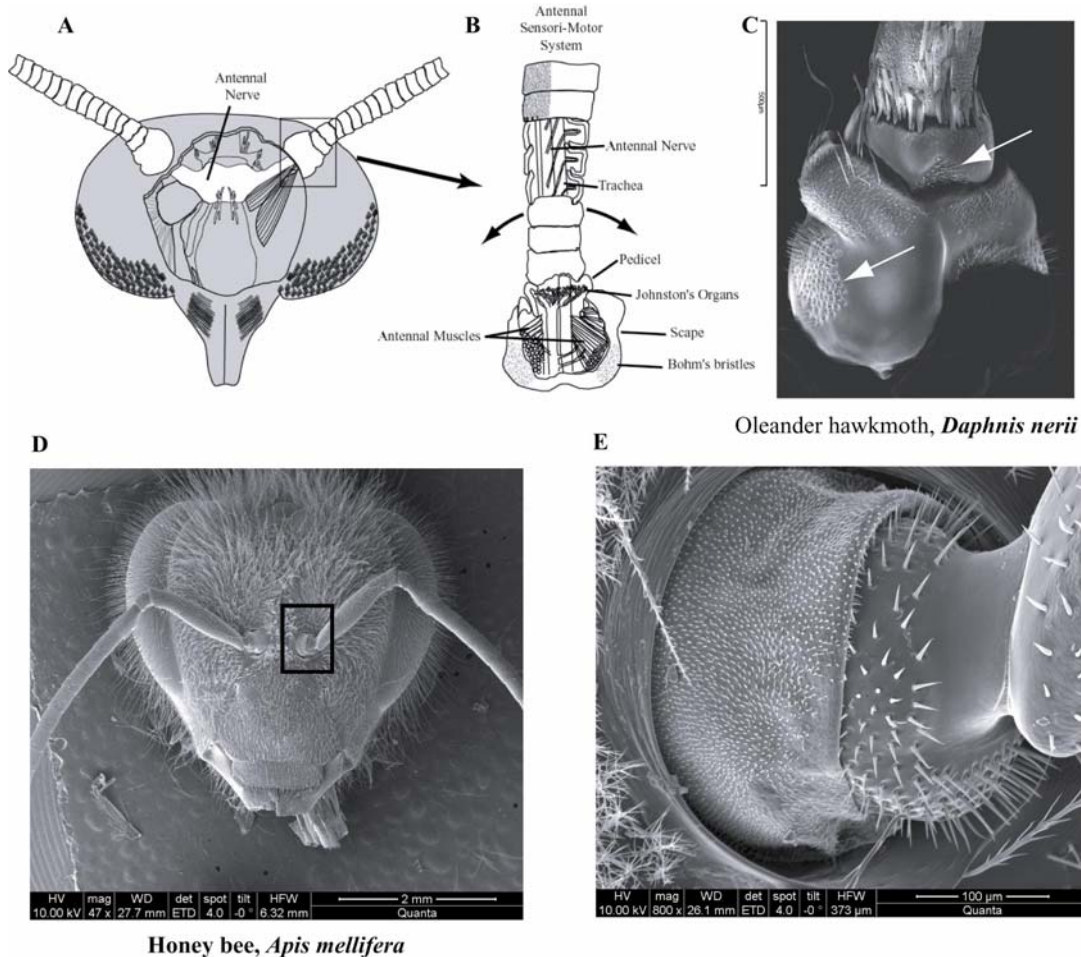


Figure 1: Antennal Anatomy (A) Frontal view of antenna and brain connected by antennal nerve. (B) Morphology of the antenna. (C) Scanning electron micrograph (SEM) of antennal base. Arrows indicate location of the Böhm's bristles. (D) SEM image of the bees head and antennae and (E) a close-in image of the antennal base with the Böhm's bristle fields.

Characterization of the antennal positioning behavior (Anand Krishnan)

Description of the antennal positioning behavior: We have developed an assay to perturb the antennal position in tethered flapping moths and used this assay to measure antennal recovery following a brief period of mechanical perturbation. These experiments show that the normal antenna rapidly and stereotypically recovers its original position when perturbed. We are presently conducting a detailed set of experiments using the above assay to describe the kinematics and dynamics of the recovery of the antennal position.

Identifying principle mechanosensory components of the antennal positioning response (Anand Krishnan). To identify the principle mechanosensors that mediate antennal positioning in moths, we conducted several experiments. First, ablation of the all Böhm's bristle fields caused the antenna to be improperly positioned during flight. Second, ablation of only the scapal Böhm's bristle fields also caused the antenna to be improperly positioned during flight. Third, the effect of ablation of pedicellar bristles appeared to be negligible. Fourth, restriction of the pedicel-flagellar joint (and thus reduction of input to the Johnston's organs) has no measurable effect on antennal positioning response indicating that Johnston's organs may not be involved mediating this response. Thus, our pilot data identifies the Böhm's bristles as the primary mediators of antennal positioning.

Neural circuitry underlying antennal sensorimotor integration (Subashini Sudarshan)

To visualize the underlying neural connectivity, we have performed neuroanatomical investigations using double-dye fills of the sensory and motor ends of the antennal system. These show an extensive overlap of the Böhm's bristle arbors with the dendritic branches of the antennal motor neurons suggesting a direct connectivity between these two systems. Based on these investigations, we propose the hypothesis that the antennal positioning reaction is mediated by the Böhm'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.

Although preliminary, these results are particularly exciting because if the antennal positioning response is a classic reflex arc, then its simple connectivity and easily measurable behavior make this a very promising preparation for many future studies due to the easy access of the mechanosensory neurons and the antennal motor neurons (both of which pass through the antennal nerve) as well as the ability to conduct these studies all the way from behavior to the neurobiology. In addition, it is also possible to conduct other studies ranging from single sensor level investigations (e.g. encoding and adaptation properties of the bristle neurons) to systems-level questions regarding the development and evolution of antennal positioning. To ascertain that this behavior is

relevant to other insects, we have looked at insects of other Neopteran orders such as Orthoptera, Hymenoptera, Mantodea and Neuroptera and confirmed that the Böhm's bristles are present across a diversity of insect orders and hence the bristles and their underlying connectivity are conserved features in all Neoptera.

Antennal positioning in bees (Taruni Roy)

We began research on bees to exploit their trainability in achieving some of the more difficult behavioral assays. We set up an apiary in August-September 2009 and 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. We get them to fly upwind while keeping track of their antennal angles and flight trajectory with two high-speed video cameras and measure how bees position their antennae during free flight.

We have also repeated several experiments that were previously conducted on moths to establish antennal involvement in flight control. From these pilot data, it appears that the bees 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.

*Location of odor sources in the fruit fly, *Drosophila melanogaster* (Nitesh Saxena)*

We assembled a system to quantify the 3D flapping movement of insect wings and body with high temporal resolution using high-speed videography. Using this system, we studied how fruit flies pinpoint the location of odor sources and observed that 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. When a fly is presented with an invisible odor source placed some distance away from a single black object, its trajectory 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 suggested 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 source from among a clutter of objects. We have lately been attempting to uncover these rules.

Recently, we tested the flies performing the above described tasks in a wind tunnel and noticed that flies behave more robustly to odor stimuli in presence of air flow. Hence we have recently begun conducting our behavioral experiments within a wind tunnel. In tandem, we are also trying to understand how flies at a distant downwind location track an upwind odor plume to arrive in the vicinity of the food source.

Landing and turning behaviors in the housefly, Musca domestica (Navish Wadhwa, Sathish Kumar): To understand how flies integrate inputs from multiple sensory modalities during landing, we have devised diverse behavioral assays to study landing and turning behaviors in flies. We film these flies to study their wing motion in great detail and we are using these data to address basic questions about sensory-motor integration and the flight mechanics of aerial turns. Presently, we are focusing on how flies perform pitch and yaw maneuvers. 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 body and wing kinematics. A quasi-steady aerodynamic model that 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.

Field studies on moth flight (Aravin Chakravarthy)

We recently undertook experiments to train the moth, *Daphnis nerii* 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. nerii* 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 (for host plant recognition by females and nectar

source recognition by males and females). Although we could elicit and film all these behaviors, their occurrence is not deterministic. Hence, we need to understand what the specific environmental conditions are which are best suited to elicit these natural behaviors. Increasingly, these experiments appear critical for future work because *Daphnis nerii* offers several attributes of a 'model' study system to study diverse questions relating to insect flight. In the coming year, we hope to investigate this system from a natural history perspective in some depth.

Software and/or Hardware: None