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14. ABSTRACT

This research focuses primarily on insect flight. We look at this phenomenon from diverse angles. These include the physical study of flapping wing flight, neuro-physiological study of how sensory information is encoded, processed and decoded to provide flight motor responses, a behavioural study of flight responses to sensory stimuli, and during rapid aerial manoeuvres, and an ecophysiological study of how multi-sensory cues are used in natural behaviours, including foraging, ovipositioning and long-distance migrations. Such an eclectic approach is necessary for a deeper understanding of the physics and biology of insect flight, its role in evolution and its influence upon ecology. In keeping with this goal, our study is not focused on any one insect system but takes on a broadly comparative approach. We use hawk moths (Lepidoptera), various types of flies (Diptera), honey bees (Hymenoptera) etc. to study diverse flight-related questions.

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Final Report for AOARD Grant 114057

“Sensorimotor Integration of Antennal Positioning in Flying Insects.”

Feb 23, 2015

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Abstract:

Research in my laboratory focuses primarily on insect flight. We look at this phenomenon from diverse angles. These include the physical study of flapping wing flight, neurophysiological study of how sensory information is encoded, processed and decoded to provide flight motor responses, a behavioural study of flight responses to sensory stimuli, and during rapid aerial manoeuvres, and an ecophysiological study of how multi-sensory cues are used in natural behaviours, including foraging, ovipositioning and long-distance migrations.

Such an eclectic approach is necessary for a deeper understanding of the physics and biology of insect flight, its role in evolution and its influence upon ecology. In keeping with this goal, our study is not focused on any one insect system but takes on a broadly comparative approach. We use hawk moths (Lepidoptera), various types of flies (Diptera), honey bees (Hymenoptera) etc. to study diverse flight-related questions.

Introduction:

The evolution of flight in insects and the miniaturization of their body size enabled insects to colonize wide-spread terrestrial niches that were inaccessible for most other animals. However, aerodynamic constraints on flapping flight demand that any reduction in wing size must be accompanied with an increase in wing beat frequency to generate requisite flight forces. In turn, the increase in wing beat frequency requires matching sensory and motor adaptations. From a physical perspective, the flapping flight wings must generate stable forces and torques around the insect body. From neural and biomechanical perspectives, aerial agility and rapid manoeuvring means that sensory perception also needs to be speedy. Not surprisingly, insect nervous systems sense and respond to their environmental stimuli much faster than larger animals. For instance, flicker fusion rates in insect compound eyes are ~100-200 Hz, some 10 times faster than humans. Multi-modal sensory inputs arriving with different latencies must elicit a rapid response e.g. mosquitoes flap at frequencies as high as 450 Hz, each wing stroke taking <3 ms matching the timescales of single action potentials.

As a result, insect flight behaviours such as take-off, landing and rapid turns occur in timescales faster than human perception, and require high-speed videography for observation and measurement.

Does the demand for speed lead to a corresponding loss of precision? Even mundane observations of flying insects such as territorial chases of insects, rapid turns, food-finding etc. are precisely coordinated in space and time, although these manoeuvres require an integration of inputs from multiple sensory modalities. Thus, when houseflies land upside down on a ceiling or moths migrate over long distances, they require visual and mechanosensory inputs to maintain proper course and flight balance. How are sensory inputs of disparate latencies integrated and compiled by the insect brain to manufacture rapid, precisely coordinated behaviours? At the level of neurons and neural networks, how are these tasks handled? How precise must these behaviours be for the survival and success of an insect species in the natural context? These are the kinds of questions we have addressed with the funding received from AOARD. On occasion, these questions have also led us to explore areas beyond the scope of the proposal which are outlined towards the end of this report.

Over the past five years, I have advised 8 graduate students and 1 Master's student.

Graduate students:
Anand Krishnan (Integrated Phd: joined 2007 - graduated 2014),
Nitesh Saxena (Integrated Phd: joined 2007 - to graduate 2015),
Tanvi Deora (Integrated Phd: joined 2008 – to graduate 2015),
Taruni Roy (Integrated Phd: joined 2008 – to graduate 2015),
Umesh Mohan (Phd: joined 2010 - to graduate 2016),
Harshada Sant (Phd: joined 2012 – to graduate 2017)
Dinesh Natesan (Phd: joined 2012 – to graduate 2017)
Payel Chatterjee (Integrated Phd: joined 2012 – to graduate 2018)
Abin Ghosh (PhD: joined 2015- to graduate 2020).

Master's student:
Sunil Prabhakar (joined 2009- graduated 2013)

Overview of Research Program

My laboratory studies the neural and physical mechanisms underlying flight behaviours in insects. We study these behaviours from diverse perspectives including aerodynamics, biomechanics, neurobiology and eco-physiology, typically choosing questions that integrate two or more approaches. In the past five years, we have sought to understand how flying insects rapidly acquire, integrate and process sensory inputs from multiple modalities (e.g. vision, mechanosensation, olfaction etc.) to generate flight behaviours, and also how wing movements generate the forces and torques around the body that translate to pitch, roll, yaw manoeuvres or combinations of these. We are interested in these behaviours during free ranging flight e.g. precise odour tracking or ovipositioning, and long-distance migrations.

At the heart of each project is a specific 'behavioural module' (e.g. landing, fast turns, take-off, antennal positioning etc.) which is clearly identifiable as a rapid stereotypic motor pattern ('manoeuvre') and can be experimentally elicited by presenting insects with appropriate sensory stimuli. We first try to recreate these behaviours in lab using specific assays, and then record and quantify the behavioural response using techniques with high temporal resolution such as high-speed videography, Laser Doppler Vibrometry etc. Manipulating behaviour by altering sensory inputs leads to various hypotheses about the underlying neural processes, which is complimented by neuroanatomy and neurophysiological experiments on whole animals. Once the main sensory-motor components are identified, we study their role in whole-animal behaviour. We hope to take these studies out into the field to study behaviour in natural circumstances.

Initially, we focused on rapid behaviours which may be considered as building blocks for more complex flight behaviours. To study these behaviours, we did not adopt any single model animal but instead developed different insect systems, including hawk moths, honey bees, house flies, fruit flies, soldier flies etc. selecting the best exemplars of behaviours of interest.

Here we describe some of our ongoing projects which benefited directly or indirectly from the funding received from the Air Force Office of Scientific Research / AOARD:

- Antennal control of flight in the oleander hawk moth, *Daphnis nerii* and honey bee, *Apis mellifera.*
- Wing-haltere coordination in the soldier fly, *Hermetia illucens*.
- Localization of odor sources in the fruit fly, *Drosophila melanogaster*
- Natural history of plant-insect interactions in the oleander hawk moth, *Daphnis nerii*
- Biomechanics of prey capture in bladderworts, *Utricularia stellaris*

Antennal control of flight in the oleander hawk moth, *Daphnis nerii* and honey bee, *Apis mellifera* (*Students: Anand Krishnan, Sunil Prabhakar, Harshada Sant, Taruni Roy, Subashini Sudarsan)*

Purposeful locomotion requires accurate sensory feedback about the consequences of one's movements. Like aeroplanes, insects also use combination of sensors to receive state feedback during flight but the transduction and processing times of each sensory modality are different, varying from a few milliseconds for certain mechanosensors to tens of milliseconds for visual sensors. For large animals such as birds, these delays still allow plenty of time to generate response. However, the speed of responses in insects must be on the order of single wing strokes or few milliseconds. How does an insect combine multimodal feedback in such rapid time scales to stabilize and control flight?

During my postdoctoral work, I had showed that mechanosensory feedback from a set of antennal mechanosensors, the *Johnston's organs,* was important for flight control. When Johnston's organs were unloaded by cutting the flagellum above the pedicel-flagellar joint, insects lost control of their flight trajectory but when they were reloaded by gluing the cut flagellum to its stub, insects regained control. We conducted intracellular recordings from single units of the Johnston's organs (the first such recordings) to understand their tuning properties and hypothesized that they encode Coriolis strains at the base of antennae, similar to halteres in Diptera (Sane *et al*., 2007). These experiments demonstrated that mechanosensory feedback from Johnston's organs is range-fractionated and capable of providing information about Coriolis strains, however the mechanistic basis of this system needs to be better elucidated (*Dieudonne, Daniel and Sane, 2014*). We are therefore using a combination of behavioural, neuroanatomical and neurophysiological investigations in a comparative framework to study how antennal mechanosensors mediate flight control

Over the past five years, we have developed two study systems - the hawk moth *Daphnis nerii* and the honey bee *Apis mellifera*. These systems differ by an order of magnitude in size and wing beat frequencies (as a measure of response time). We are using these systems to study the combined role of mechanosensors and vision in flight. We first investigated the antennal positioning behaviour which occurs at the onset of every flight bout in moths and bees in both tethered and free-flight conditions. In one assay, we perturbed the antennal position in moths and monitored its rapid recovery using high-speed videography. Using this assay, we identified the primary mechanosensory structures, called *Böhm's bristles* that stimulate the antennomotor system to recover from antennal perturbations. Our neuro-anatomical investigations suggested that the underlying sensory and motor neurons are directly connected, and hence the antennal positioning response is a classic reflex system (Figure 1). Neurophysiological studies showed that the latency of sensory stimulation to motor response is indeed very short (< 10 ms). Thus, antennal positioning response may be a monosynaptic reflex (Krishnan et al, 2012). Clearly, one way in which an insect brain handles the need for fast responses is by ensuring more direct connectivity of neurons.

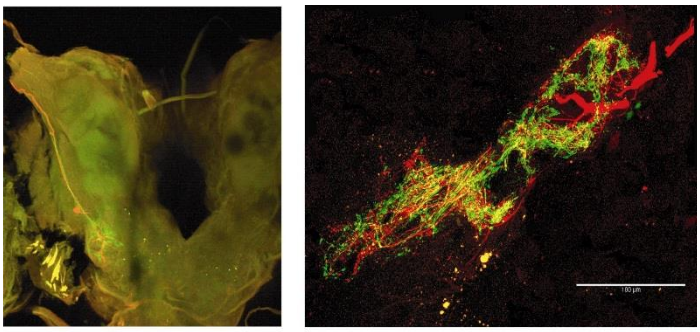

Figure 1 .Arborization patterns of sensory-motor neurons. Double dye fills of the Böhm's bristle arbors (green) and antennal motor neurons (red). The two overlap in the Antennal Motor and Mechanosensory Center (AMMC) of the brain. Whole brain view (Left panel) and close-in view of the arbors (Right panel)

Visual inputs to antennal positioning: When flight is elicited with looming visual stimuli, it invariably elicits rapid antennal positioning (Fig 2A). Electromyogram recordings from antennal motor neurons show correlated changes in antennomotor activity in response to looming stimuli (Fig 2B). Moreover, tethered moths alter their antennal position with changing optic flow. In neurophysiological recordings, antennal muscle activity patterns to depend on visual feedback. We therefore hypothesized that visual inputs provide feedback to the antennal positioning apparatus (Fig 2C).

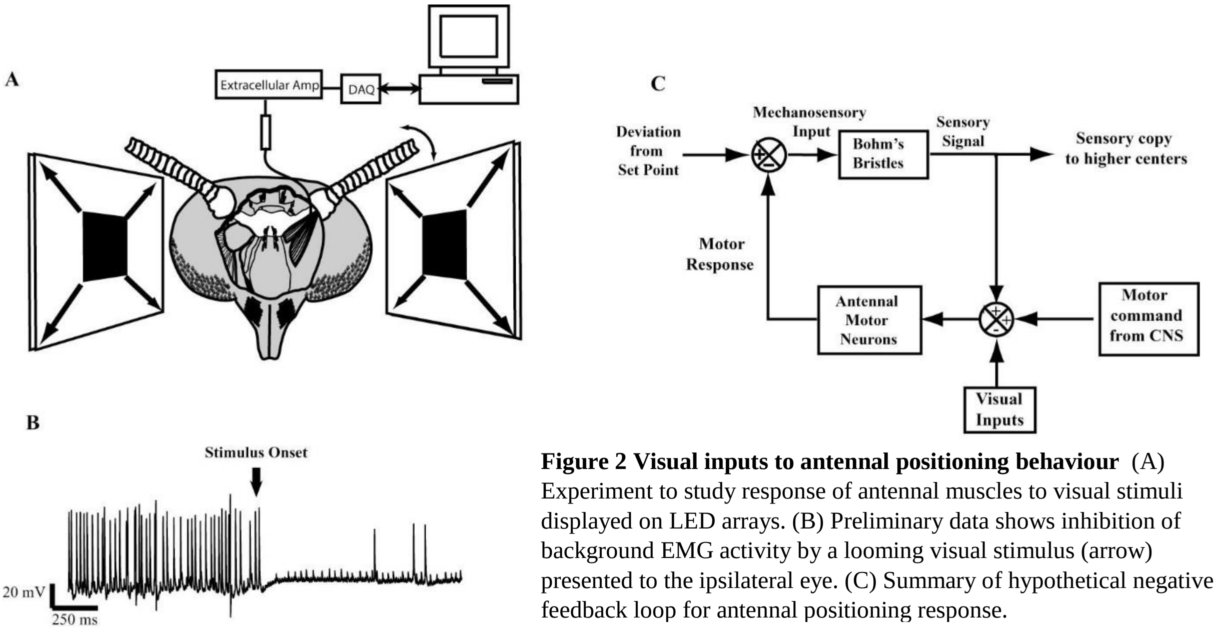

Figure 2 Visual inputs to antennal positioning behaviour (A) Experiment to study response of antennal muscles to visual stimuli displayed on LED arrays. (B) Preliminary data shows inhibition of background EMG activity by a looming visual stimulus (arrow) presented to the ipsilateral eye. (C) Summary of hypothetical negative feedback loop for antennal positioning response.

This was established using two experiments. First, antennal muscles of both ipsi and contralateral antennae respond to an LED light pulse on an eye with latencies around 50 ms. Second, antennae respond in a direction-specific manner to moving visual stimuli suggesting that they receive directional information from the eyes. Thus, antennal positioning is also multi-modal, receiving inputs from both vision and mechanosensation (Krishnan and Sane, 2014).

In a parallel set of studies, we set up an apiary and trained honey bees to fly through a wind tunnel to access a nectar feeder. We adjusted the air flow in the wind tunnel and filmed the bees as they positioned their antennae in relation to the air speed during free flight. We also conducted experiments on tethered bees in the wind tunnel. In both cases, the antennal position is a function of air speeds, and not ground speeds of the freely-flying bee. This result confirms that flying insects can sense their air speed relative (Fig 3; Roy and Sane, *in prep*), and future studies are required to identify the mechanosensor that detects airflow and mediates antennal positioning in bees and moths.

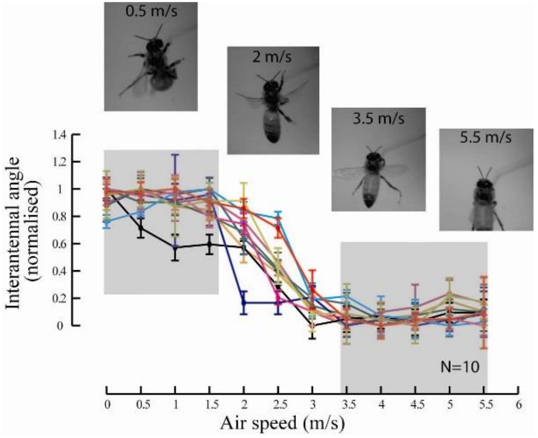

Figure 3: Inter-antennal angles in a tethered bee. (Top staggered panels) Sequence of images of a tethered bee as it changes its inter-antennal angles with air speed. (Bottom panel) Inter-antennal angle as a function of air speed. These experiments were carried out on tethered bees in a wind tunnel. Inter-antennal angles calculated from the digitized high-speed videos clearly show that they decrease as the air speed increases from 1.5 m/s to 3.5 m/s. For air speeds less than approx. 1.5 m/s and more than approx. 3.5 m/s, they do not significantly alter (grey boxes). The resulting function resembles a sigmoid relationship typical of sensors.

Publications:

Krishnan, A; Prabhakar, S.; Sudarsan, S. and Sane, S. P.* (2012) Neural mechanisms of antennal positioning in flying moths. **Journal of Experimental Biology 215, 3096-3105**

Krishnan, A. and Sane, S.P.* (2014) Visual feedback influences antennal positioning in flying hawk moths. **Journal of Experimental Biology 217, 908-917**

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

Mechanisms of coordination of wings and halteres in Dipteran insects

(Tanvi Deora, Shilpa Naik, Amit Singh, Nehal Johri and Kumar Vardhanam Daga ; collaborators: Sufia Sadaf, Gaiti Hasan, Rizwana Parween, Rudra Pratap and Namrata Gundiah from IISc. Mechanical Engineering)

For insects with miniature body forms, aerodynamic laws demand that their wings flap at elevated frequencies often exceeding 100 strokes/second, leaving little time for the nervous system to sense and respond to sudden aerial perturbations on a stroke-to-stroke basis. This is especially true in insects such as fruit flies flapping at 250 Hz or midges reportedly flapping at 1000 Hz. In these Dipteran insects, the halteres (hind wings of flies which evolved into gyroscopic mechanosensory structures) provide key mechanosensory inputs for flight stability by providing feedback about the angular velocity of their body during flight. Halteres move with a precise phase relationship to the wing motion which appears to be crucial for stable flight, because even the slightest error in wing-wing or wing-haltere coordination can cause instability during flight. How do flies and other insects cope with the challenge of being both fast and precise?

To address this question, we began investigating the neural and biomechanical basis of the wing-haltere coordination in the soldier fly, *Hermetia illucens*. We initially sought to test the hypothesis that wings and halteres were coordinated through active nervous control. Much to our surprise, such coordination could be elicited in dead flies, where the nervous system is inactive. This led us to a series of investigations that explored the thoracic architecture and biomechanics, rather than the nervous control.

Through systematic investigation of wing and haltere motions, we showed that the thorax contains a series of mechanical linkages that directly govern coordination of the wings and halteres. In addition, we proposed the existence of a novel *wing clutch,* along with a previously described *gearbox* that enables the wings to link or delink from the thoracic linkage network, giving the flies a measure of independent control over each wing. The model of the insect thorax emerging from these studies not only explains wing-haltere coordination, but applies to flies of all species, with obvious implications for studies in other insects with miniaturized body forms. To model the mechanics of halteres and wings, we are collaborating with Prof. Rudra Pratap's group and Dr. Namrata Gundiah's group at the Indian Institute of Science.

If there is a separate clutch on each side of the thorax, then there must be an underlying mechanism to coordinate the engagement of both clutches during the onset of flight. We are collaborating with Prof. Gaiti Hasan and her graduate student Sufia Sadaf who are using the tools of *Drosophila* genetics to address the role of aminergic neurons in setting up the flight circuitry. These studies indicate that RNAi-based knockdown of the STIM (the Ca^{+2} sensor on endoplasmic reticulum) expression in *Drosophila* dopaminergic neurons causes significant flight defects. Although these flies show normal wing posture, they are unable to sustain flight for long duration. In cases when such flies can fly, their wing beat frequency is normal but the coordination of right and left wings is defective perhaps due to improper the synchronization of bilateral wing engagement. This hypothesis led us to look for the dopaminergic neurons in the central nervous system that drive bilateral coordination of both wings, the loss of function in which causes improper flight coordination. Recently, we have identified a single Ventral Unpaired Median (VUM) neuron that appears to feed into the b1 motor neurons (neurons driving one of 17 steering muscles involved in stroke positioning) of both right and left sides, which seems to be a likely candidate for driving coordination of bilateral wing engagement or disengagement. Without the clutch hypothesis emerging from our solider fly work, it is unlikely that we would have focused on wing engagement *per se*. These experiments showcase how comparative research can provide insights useful for work in model systems such as *Drosophila*.

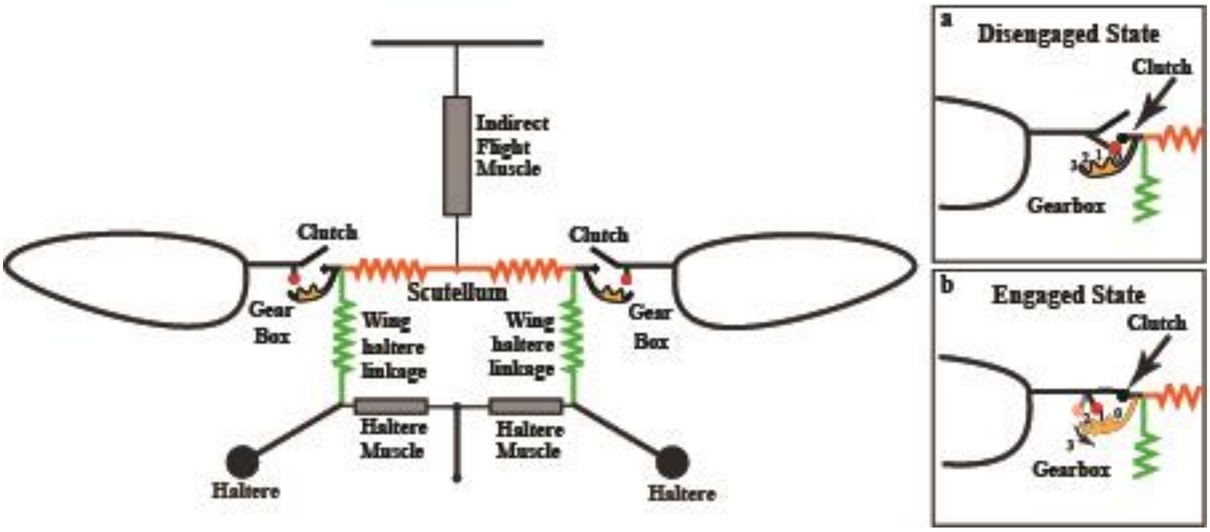

Fig 4: A mechanical model of the Dipteran thorax. Model shows the mechanical connections that are responsible for wing-wing coordination and also wing-haltere coordination. A clutch and gearbox mechanisms helps with independent control of each wing.

Publications:

Deora, T, Singh, AK and Sane, SP* (2015) Biomechanical basis of wing and haltere coordination in flies. **Proceedings of the National Academy of Sciences, 201412279**

Sadaf, S, Reddy OV, Sane, SP and Hasan, G* (2015) Neural control of wing coordination in flies. **Current Biology 25 (1), 80-86**.

Parween, R*; Pratap, R.; Deora, T. and Sane, S.P. (2014).Modeling strain sensing by the gyroscopic halteres in the Dipteran soldier fly, Hermetia illucens. **Mechanics Based Design of Structures and Machines 42 (3), 371-385**

Navigational strategies for odor source localization *(Nitesh Saxena, Dinesh Natesan)*

What general rules govern how insects locate odor sources? We have developed a system of three high-speed video cameras to film and quantify the detail 3D movement of the fly's wings and body with high temporal resolution (Fig 5A, B). Because bright light hinders natural behaviour, we filmed in infrared (IR) light which is invisible to fruit flies thereby ensuring 'naturalistic' insect behaviour. First, we explored the decision-making process in the moments before a fly identifies an odor source. We found that in the presence of a single, visible odor source, flies punctuates their rapid approach with a distinct hovering phase some distance above the object, followed by rapid descent as the fly lands on the odor source (Fig 6 A-C). If the flies encounter 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 6 D-F). In our experiments, *Drosophila melanogaster* flies were introduced in a wind tunnel test section uniformly illuminated by an array of IR LEDs. At the upwind end of the wind tunnel, we place an odor source, typically apple cider vinegar to which they are attracted, in a transparent thin capillary.

To understand how flies used both visual and olfactory information to pinpoint the location of odor sources, we ran two kinds of experiments. First, we topped the odor capillary (Odor Object) with a black bead (Visual Object) that served as a high-contrast visual stimulus against the white background. We then assayed the ability of *Drosophila melanogaster* to land on the odor source at variable separations between the odor object and the visual object. Second, we presented the flies with 3 or 7 visual objects ('clutter'), one of which was coupled with the odor source. The fly must then find the odor object amidst the clutter. When a fly is presented with an invisible odor source placed some distance away from a single black

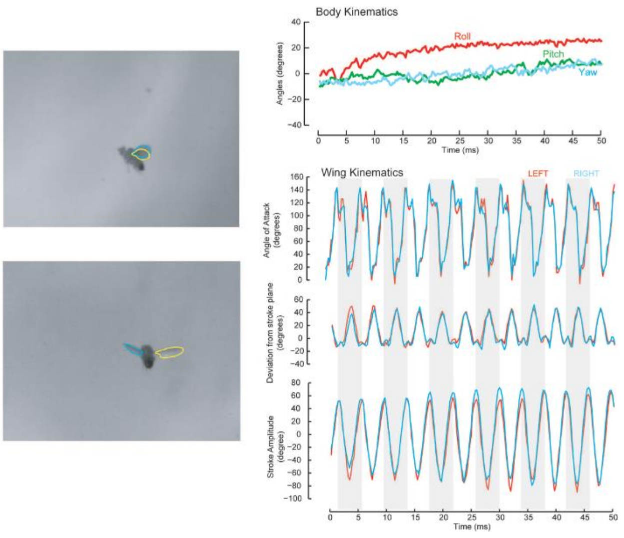

Figure 5: Extracting wing and body kinematics from freely flying *Drosophila melanogaster*. (A) Two frames, each showing the silhouette of the adult fly from different angles. Virtual wire frame models enable an exact reconstruction of the wing position at every time point and the head and tail can be 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.

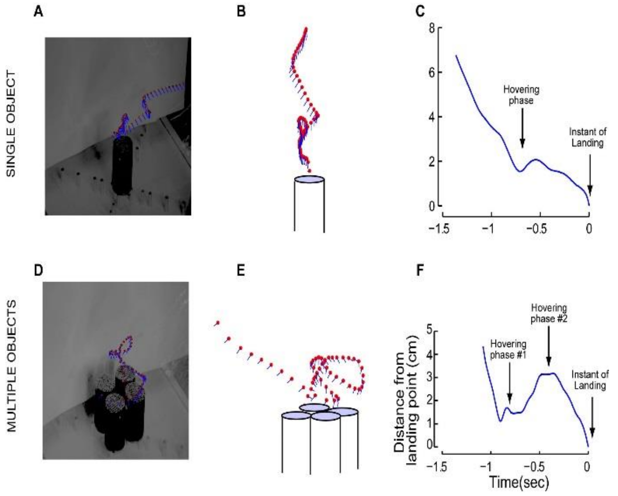

Figure 6: Odor tracking in freely flying *Drosophila melanogaster*. (A-C): Fly tracking a single visual and odor object. **(D-F):** Fly tracking a single odor object but multiple visual objects. **(A, D):** Frames from one of the high-speed cameras. The fly's trajectory is depicted as a ball and stick 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 when tracking a single object **(C)** and multiple hovering phases when tracking multiple objects **(F).**

object, its trajectory is largely confined to the volume between the odor source and the visual object, suggesting that it uses both cues when making navigational decisions. These experiments have yielded great amounts of data which we are currently analyzing in detail using a variety of statistical techniques. Our results indicate that flies are most effective at finding odor sources when the odor source spatially overlaps with the visual object. If there is a separation between the odor and visual objects, flies tend to make mistakes in identifying the odor source.

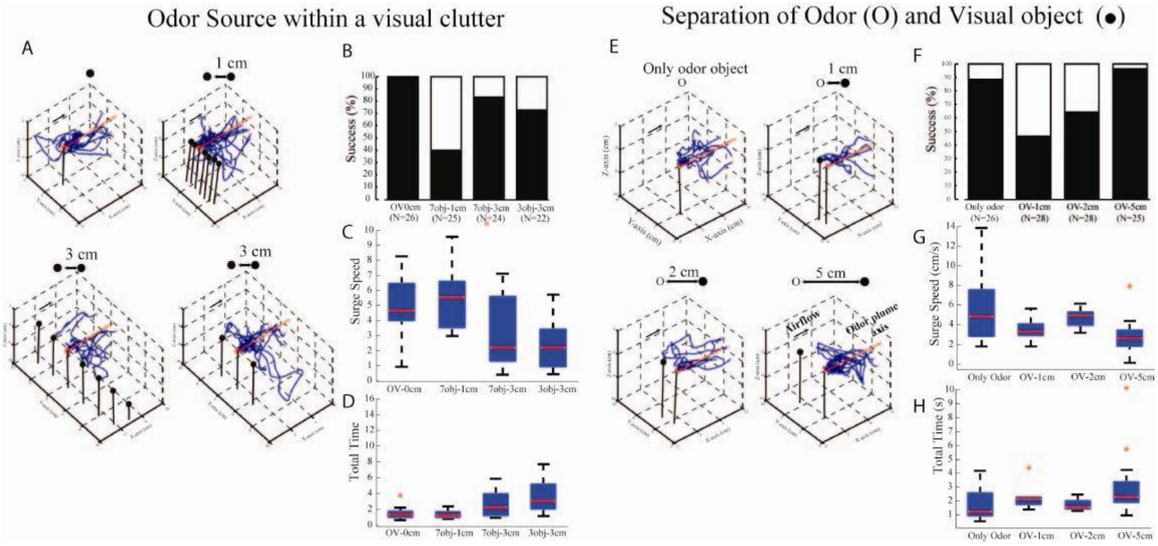

Figure 7: Responses of flies to odor and visual stimuli (A) Trajectories of flies locating the odor object within a clutter of visual objects. Red line indicates the direction of plume as measured using smoke visualization, and black arrow indicated the direction of air flow in the wind tunnel. From these data, we can measure several parameters such as (B) success rate of odor source localization, (C) surge speed (in cm/s) and (D) total time taken (seconds) for a fly that enters with an 8 cm sphere around the odor source to locate the odor source. (E) Trajectories of flies locating the odor object associated with a visual object or some distance away from it. (F) Success rate (G) surge speed (in cm/s) and (H) total time taken (seconds).

Taken together, our results strongly suggest that the fly consolidates information from both visual and olfactory inputs before making landing decisions. If this is true, 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 write these results after completing the analysis of all the data over the next 4-5 months (Fig 7A-H; *Saxena, Natesan and Sane, in prep*).

In addition to these complex behaviours, we have also been able to elicit simple behaviours such as pitch, roll and yaw turns and also landing in houseflies, which we are separately trying to understand. In these experiments, we have quantified the wing kinematics during these manoeuvres in detail thus providing insights into how these insects manufacture simple turns *(Natesan, Wadhwa and Sane, in preparation; Saxena, Natesan and Sane, in preparation*)

Natural history of plant-insect interactions in the oleander hawk moth, *Daphnis nerii (Ajinkya Dahake, Aravin Chakaravathi)*

From an evolutionary standpoint, flight enables insects to escape from harsh to more conducive climes for their survival and breeding. One spectacular annual manifestation of this occurs when millions of butterflies and moths migrate over long distances. We are interested in the following broad questions relating migration to insect-plant interactions:

1. How do small insects, with a limited fuel supply, undertake such long journeys?
2. What are the local and global sensory guidance cues are used for migration?
3. What physiological changes do they undergo to sustain long-distance flight?
4. How does host plant phenology correlate with insect development and migration?

We are interested in the physiological processes, rather than patterns, of migration. However, to study the physiology of migration, it is important to first know the patterns of insect migration to identify good study systems. In a neo-tropical diurnal moth *Urania fulgens* that migrates over the Panama Canal, we investigated how antennae provide local guidance cues for long-distance navigation and showed that *Urania* antennal mechanosensors are required for proper orientation during migratory flight, but not in determining airspeeds of the insect. Flight-capable *Urania* maintained airspeeds in absence of antennae, indicating that a sensory organ other than antennae is involved in air speed regulation. As in locusts, a set of bristle structures called the cephalic hair may be involved in measuring air flow over the body (Sane et al, 2010).

Although the Indian subcontinent is rich in migratory species and habitat diversity, we know surprisingly little about patterns of Lepidoptera migration in India. Since the last major butterfly migration survey in pre-independence India (Williams, 1937), only a few (mostly anecdotal) accounts of Lepidopteran migration in India have been published. To address this lacuna, we began sampling hawk moths at an NCBS field site in Agumbe in the Western Ghats since 2012. Parallel sampling will be conducted at other sites, including the Andaman Islands and Pachmarhi in Central India. Our initial ‘modest’ goal was to sample monthly over two years to identify seasonal patterns of abundance that may indicate migration events. In the process, we will generate a catalog of Indian hawk moths as the bare minimum deliverable outcome. This effort is now two years old and we have fine-tuned our sampling techniques sufficiently to begin building a database of hawk moths in the Western Ghats. Currently, we have identified over 45 hawk moth species in just Agumbe.

We have also developed a greenhouse facility on campus to study plant-insect interactions, specifically to address the hypothesis that migration in moths (and other Lepidoptera) is resource-driven and linked to the host plant phenology, fuelled by their need to find fresh leaves for egg-laying. Our exploratory studies to understand how the Oleander hawk moth, *Daphnis nerii* identifies her host plants, *Nerium oleander* and *Tabernae montana divaricata* are yielding interesting results. In the the *Daphnis- Nerium* system, larvae emerging from eggs deposited on older leaves do not survive, either because newly emerged larvae lack the mouthparts to eat the tough cuticle of the older leaves, or because these leaves are too toxic. Egg-laying moths (and butterflies) must thus possess the ability to distinguish older leaves from younger leaves to ensure that their offspring survive. This simple hypothesis is powerful because it relates the physiology of insects (feeding and oviposition habits) to the pattern of migrations (leaf phenology) in a field-testable manner.

We filmed *Daphnis nerii* in an insectary as they identified host plants for ovipositioning, and quantified their ability to estimate leaf maturity. We observed that *Daphnis nerii* could accurately distinguish between new and old leaves in a plant with mixed leaves. Our pilot experiments show that they use both olfactory and visual cues in this task. Larvae hatching on mature leaves explored the leaf margins before moving towards the stem and climbing towards the apex of the plant to access fresh leaves. These initial studies provide fascinating insights into the natural history of the *Daphnis nerii-Nerium Oleander* interactions which we will explore in greater detail in the coming years. A paper describing our data is currently being written up (*Chakravarthy, Dahake et al in preparation*).

Publications

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

Manuscripts in preparation:

Chakravarthi, A., Dahake, A.S. and Sane, S.P. Ovipositioning behaviour in the Oleander hawk moth, Daphnis nerii (in preparation)

Biomechanics of prey capture in aquatic bladderworts, *Utricularia stellaris(Amit K. Singh, Sunil Prabhakar*)

A small part of my laboratory studies the biomechanics of plant-insect interactions. Although not directly related to flight, these modular projects help us develop tools for specific measurements of plant-insect interactions, while also providing mechanistic insights into such interactions. In one such project, we investigated how the aquatic bladderworts, *Utricularia stellaris*, although lacking a nervous system, captured insect prey. Bladderworts prey upon swimming insect larvae or nematodes to supplement their nitrogen intake. The closed *Utricularia* bladder, a modification of its leaf, develops lower-than-ambient internal pressures in their bladder walls by pumping out water from the bladder. The resulting elastic instabilities in their bladder walls mean that bladder walls can snap outward causing large suction pressures at their mouth. We first developed a lab assay wherein we could get predictable prey capture events, using either mosquito larvae or finely-drawn glass pipettes. We then filmed the prey capture using high-speed cameras at 10,000 fps. When mechanically stimulated by moving prey or capillary, the external sensory trigger hairs on the *Utricularia* trapdoor are activated causing the trapdoor to open within 300–700 μs, causing strong suction flows that overwhelm and trap their prey. The trapdoor opening is perhaps the fastest recorded motion in carnivorous plants. We were also able to design custom-probes to measure the internal pressures of the *Utricularia* bladders. These values allowed us to predict suction flows and match them against the measured flows. Thus, we were able to investigate the mechanisms of prey capture in aquatic bladderworts, *Utricularia stellaris.*

Through the two above-described studies, we have begun exploring plant-insect interactions on a broader level. I see this as an area that is critical to explore over the coming years because it combines knowledge of insect behaviour with chemical ecology and provides a whole new dimension to neuroethology

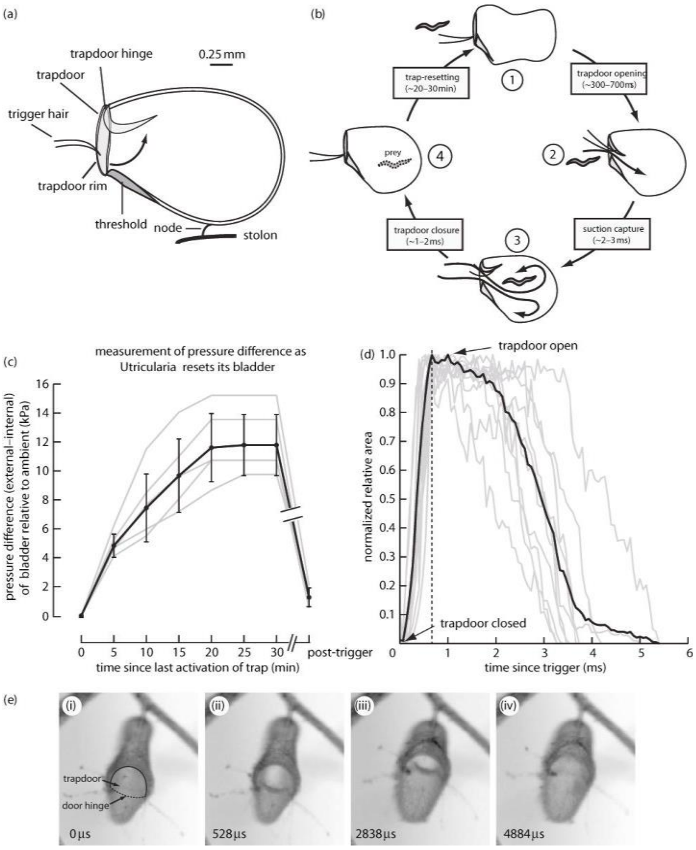

Figure 8. Opening and closing of the trapdoor of *U. stellaris* and related flows. (a) Diagram of *U. stellaris* bladder. Arrow shows opening of the trapdoor. (b) Prey capture in *U. stellaris* including measured time scales. After an unsuccessful capture, the trap resets in ~20–30 min (1). When trigger hair are mechanically stimulated by prey, the trap door opens in 300–700 µs (2) to generate strong suction flows that capture (3) and digest (4) prey. (c) Trap-resetting phase. Plot shows how closed bladder generates lower than ambient internal pressures. X-axis shows time elapsed since last successful activation of trapdoor and Y-axis indicates external (higher)–internal (lower) pressure of bladder. Break in axis and plots indicate post-stabilization of internal pressure, the trap can be retriggered after any arbitrary time duration to bring it back to zero state. (d) Opening and closing trapdoor kinematics shown as ratio of area of open mouth to a reference rim area with time. (Grey lines) 10 individual plots normalized to maximum ratio of areas (a fully open trap), (dark black line) normalized average curve of these 10 plots. Dashed black line shows the average time point of bladder opening. (e) Four frames from a video capture show *U. stellaris* trapdoor in the following stages: (i) initiation of opening, (ii) fully open, (iii) before closing, and (iv) fully closed. Numbers in the lower left corner of each figure indicate time (□s) since initiation of door opening. The door hinge (dotted line) and margin (continuous) are labelled in panel (i). Typical diameter of trap door is 0.8 mm (*from Singh et al, 2011*)

Publications:

Singh, A.K, Prabhakar, S. and Sane, S. P.* (2011). The biomechanics of fast prey capture by aquatic bladderworts. Biology Letters, 7, 547-550.

(This paper was mentioned by the editor-in-chief as one of five notable papers published in Biology Letters in 2011(see Charlesworth (2012) Biol. Lett. 8, 1-2))

Sane, S.P.* and Singh, A.K. (2011) Water movement in vascular plants - a primer. Journal of the Indian Institute of Science 91 (3): 233-242

List of Publications and Significant Collaborations that resulted from your AOARD supported project (all publications benefited from funding, underlined ones were directly relevant to the grant):

- **Deora, T, Singh, AK and Sane, SP* (2015)** Biomechanical basis of wing and haltere coordination in flies. **Proceedings of the National Academy of Sciences, 201412279**
- **Sadaf, S, Reddy OV, Sane, SP and Hasan, G* (2015)** Neural control of wing coordination in flies. **Current Biology 25 (1), 80-86**.
- **Dieudonne, A, Daniel, TL and Sane, SP* (2014)** Encoding properties of the mechanosensory neurons in the Johnston's organ of the hawk moth, *Manduca sexta*. **Journal of Experimental Biology 217, 3045-3056**
- **Parween, R*; Pratap, R.; Deora, T. and Sane, S.P. (2014)**.Modeling strain sensing by the gyroscopic halteres in the Dipteran soldier fly, *Hermetia illucens*. **Mechanics Based Design of Structures and Machines 42 (3), 371-385**
- **Krishnan, A. and Sane, S.P.* (2014)** Visual feedback influences antennal positioning in flying hawk moths. **Journal of Experimental Biology 217, 908-917**
- **Truong, TQ; Phan, VH; Sane S.P. and Park, HC* (2014)** Pitching moment generation in an insect-mimicking flapping-wing system. **Journal of Bionic Engineering 11 36–51**
- **Cheng, B; Sane, S.P.; Barbera, G.; Troolin, D.R.; Strand, T. and Deng, X* (2013)** Three-dimensional flow visualization and vorticity dynamics in revolving wings. **Experiments in Fluids 54:1423.**
- **Krishnan, A; Prabhakar, S.; Sudarsan, S. and Sane, S. P.* (2012)** Neural mechanisms of antennal positioning in flying moths. **Journal of Experimental Biology 215, 3096-3105**
- **Sane, S.P.* and Singh, A.K. (2011)** Water movement in vascular plants - a primer. **Journal of the Indian Institute of Science 91 (3): 233-242**
- **Zhao, L.; Deng, X* and Sane, S.P.* (2011)**. Modulation of leading edge vorticity and aerodynamic forces in flexible flapping wings. **Bioinspiration and Biomimetics 6 (2011) 036007.**
- **Singh, A.K, Prabhakar, S and Sane, S. P.* (2011).** The biomechanics of fast prey capture by aquatic bladderworts. **Biology Letters, 7, 547-550.**
- **Sane, S. P. (2011).** Steady or Unsteady? Uncovering the aerodynamic mechanisms of insect flight. **Journal of Experimental Biology, 214, 349-351**

Lectures / Interactions (National and International)/ Conferences

Title: ***Inner workings of insect flight mechanisms.*** *Invited speaker,* **Institute of Advanced Study, Hong Kong University of Science and Technology** *(Dec 1-3, 2014)*

*Title**: Fast yet Balanced: how flying insects deal with the challenge of miniaturization*** Invited speaker, **RCAST, The University of Tokyo, Japan** *(August, 2014)*

Title: ***Fast, Small -- yet Still in Control: How Flying Insects Balance speed and Accuracy***

Invited Speaker: **Department of Mechanical Engineering, Purdue University, West Lafayette, Indiana, USA** *(April 11, 2014)*

Title: ***Fast, Small -- yet Still in Control: How Flying Insects Balance Speed and Accuracy***

Invited Speaker: **Department of Biology, Case Western Reserve University, Cleveland, Ohio, USA** *(April 8, 2014)*

Invited Speaker: **Booz Allen Hamilton Distinguished Colloquium series, Department of Electrical Engineering and Computer Sciences, University of Maryland, USA** *(April 4, 2014)*

Title: ***Synching the wing beat: how flies coordinate their wings and halteres***

Invited Speaker: **Systems Neuroscience Seminar, Howard Hughes Medical Institute, Janelia Farm Campus,** *(April 2, 2014)*

Title: ***Stealth of the Bladderwort***

Invited Speaker: **Symposium on Turbulence, Bangalore** *(July 20, 2014)*

Title: ***How flexible wings influence flows over flapping wings***

Invited Speaker: **International Union of Theoretical and Applied Mechanics international conference, Bangalore, India** *(January 21, 2014)*

Title: ***Looking beyond the nose: integration of multi-sensory information for odor tracking in insects***

Invited Speaker: **University of Bourgogne, Dijon, France (February 2014)**

Title: ***Fast, Small, yet still in Control: how flying insects balance speed and accuracy.***

Invited speaker: **Australian National University, Canberra (24 October, 2013)**

Invited speaker: **Indian Institute of Technology, Kanpur (28 September, 2013)**

Invited speaker: ***International Conference on Intelligent Unmanned Systems, Jaipur, India (26 September 2013)***

Invited speaker: **Georgia Institute of Technology, Atlanta (16 May 2013)**

Title: ***Looking beyond your nose: Integration of multi-sensory information for odor tracking in insects.***

Invited speaker: ***India-UK Scientific Seminar (Biology of Tropical Pollination: from individuals to Networks), Trivandram (Feb 20, 2013)***

Title: ***Fast, Small, yet still in Control: a long view of insect flight***

Keynote speaker: **Joint EuroSpin - NeuroTime Workshop, Schloss Beuggen, Germany**

Title: ***The mechanics and neurobiology of fast reflexes in flying insects***

Invited Speaker: **Symposium: Insect flight and migration, International Conference of Entomology, Daegu, South Korea** *(August 19-24, 2012)*

Title: ***The mechanics and neurobiology of fast reflexes in flying insects***

*Invited Speaker:***1st Annual Ramanujan Meeting, Organized by IISER Pune and Department of Science and Technology, Government of India, Pune** *(May 4 -6, 2012)*

*Title****: The physical and Neural basis of insect flight***

Invited speaker, **Center for Excellence in Basic Sciences, University of Mumbai-DAE,**

Kalina Campus, Mumbai *(March 27, 2012)*

Title: ***The mechanics and neurobiology of fast reflexes in flying insects***

Plenary speaker, **EuroSpin Workshop, NCBS,** *(Nov 30, 2011)*

Title: ***The mechanisms of fast sensorimotor responses in flying insects***

Plenary speaker, **Insect Flight Symposium, CAnMove program, Lund University, Sweden** *(Aug 29, 2011)*

Title: ***Mechanisms of fast sensorimotor responses in insect flight control.***

Invited Speaker: **Eglin Air Force Base, US Air Force, Florida, USA** *(19 April, 2011)*

Invited Speaker: **Wright-Patterson Air Force Base, Dayton, Ohio, USA** *(21 April, 2011)*