AFRL-AFOSR-VA-TR-2021-0075

Mechanosensory and visual integration for fly takeoff and flight

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07/15/2021
Final Technical Report

DISTRIBUTION A: Distribution approved for public release.

Air Force Research Laboratory
Air Force Office of Scientific Research
Arlington, Virginia 22203
Air Force Materiel Command

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
15-07-2021	Final	01 Apr 2016 - 31 Mar 2021

4. TITLE AND SUBTITLE
Mechanosensory and visual integration for fly takeoff and flight

5a. CONTRACT NUMBER

5b. GRANT NUMBER
FA9550-16-1-0165

5c. PROGRAM ELEMENT NUMBER
61102F

6. AUTHOR(S)
Jessica Fox

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
CASE WESTERN RESERVE UNIV CLEVELAND OH
10900 EUCLID AVE
CLEVELAND, OH
US

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
AF Office of Scientific Research
875 N. Randolph St. Room 3112
Arlington, VA 22203

10. SPONSOR/MONITOR'S ACRONYM(S)
AFRL/AFOSR RTB2

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
AFRL-AFOSR-VA-TR-2021-0075

12. DISTRIBUTION/AVAILABILITY STATEMENT
A Distribution Unlimited: PB Public Release

13. SUPPLEMENTARY NOTES

14. ABSTRACT
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15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			PATRICK BRADSHAW
U	U	U	UU	8	**19b. TELEPHONE NUMBER** *(Include area code)* 425-8492

Standard Form 298 (Rev.8/98)
Prescribed by ANSI Std. Z39.18

Final report for FA9550-16-1-0165
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29 June 2021

Abstract

We set out to describe the mechanisms of sensory information processing as used for fly takeoff and flight. Specifically, we focused on the mechanosensory halteres, the reduced hindwings of flies that are essential for flight control. In Aim 1, we quantified the role of the halteres in takeoff and flight for multiple species. It was previously known that halteres are essential in all flies for stabilizing flight, and our work significantly expanded the known roles for halteres to include gravity perception and takeoff stabilization. In Aim 2, we demonstrated that haltere stimulation changes the firing rate of neurons in the central complex, showing for the first time that haltere sensory input reaches the brain. In Aim 3, we showed that simultaneous haltere and visual stimulation has specific effects on the firing rates of central complex neurons, suggesting mechanisms for multisensory integration for long-term behaviors. This project produced eleven peer-reviewed publications and thirteen published abstracts, and the PI gave thirteen invited presentations. Current and future work focuses on understanding sensory input to the brain in multiple behavioral contexts.

Introduction

In the funded YIP award, I proposed the following hypothesis and specific aims:

Central hypothesis: Information from mechanosensory halteres and the visual system is integrated in the brain's central complex, and algorithms for this integration can change with changes in behavior.

Specific aims to test this hypothesis:
Aim 1: Quantify the role of halteres during various fly behaviors, including takeoff and flight.

Aim 2: Determine how haltere information is represented in the central complex during different behaviors.

Aim 3: Use computational neuroscience methods to determine how haltere and visual information are combined in the central complex during different behaviors.

At the close of the project, we have completed Aims 1 and 2 and are expanding our work on Aim 3. We have published data resulting from all three aims and all three aims have been more productive than initially anticipated, resulting in multiple publications and presentations.

Outcomes of Aim 1

The halteres of flies are a defining characteristic of the order, whose name itself ("Diptera") translates to "two wings." With the minor exceptions of some flightless species, all dipteran insects possess one pair of broad lift-generating front wings and one pair of small, club-shaped sensory halteres. It has been known for hundreds of years that halteres are essential to fly flight, despite the fact that they generate almost no appreciable lift (Yarger and Fox, 2016). However, it was not known whether halteres may act as sensory structures to organize other behaviors like takeoff, walking, or stabilizing the body against perturbations.

We sought to quantify the role of halteres during various fly behaviors. In several experiments, we ablated the halteres of multiple species of fly and observed the effects on behavior. In other experiments, we manipulated halteres in repeatable and quantifiable ways to examine the effect of haltere perturbations on wing and head movements. We tested flies in several different scenarios; results are detailed below.

Flight: We ablated the halteres of fruit flies and measured the effects on head movement behavior, demonstrating that haltere ablation limits flies' ability to modulate their gaze control when the stimulus is moving at high speeds (Mureli et al., 2017). We next asked how specific movements of the halteres might influence head movements, and developed an experimental arena in which we glued a small iron filing to the haltere and moved it by applying an alternating electromagnetic field. These movements were highly repeatable and allowed us to change the haltere's amplitude (Rauscher and Fox, 2021) and frequency (Rauscher and Fox, in revision). We found that input from the halteres is summed in a linear fashion to drive wing-steering movements, but that vision is a primary driver of head movements (Fig. 1).

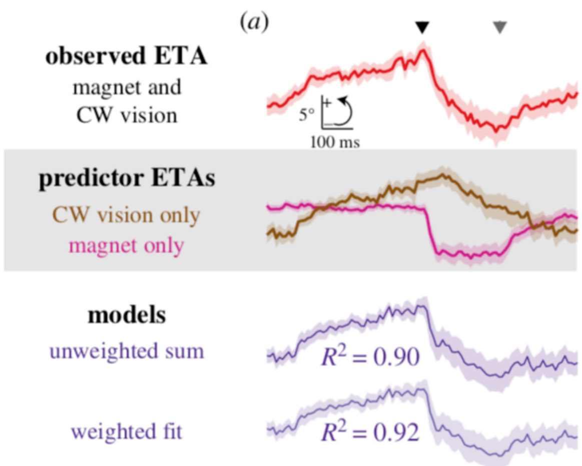

Figure 1. Event-triggered averages (ETAs) of wing-steering responses to a visual stimulus and a amplitude change to the ipsilateral haltere induced via electromagnet. The unweighted linear sum of the responses to simulations in each modality (the predictor ETAs) provides an accurate model for the observed ETA, and weighting the model does not increase the model's accuracy. This shows that the fly's wing-steering responses are effectively a linear combination of the two modalities.

Gravity perception: We placed flies in clear rectangular boxes suspended by an electromagnet. When the flies were standing on the wall of the box, we removed power to the electromagnet and dropped the box onto a substrate, creating a scenario in which flies experienced sudden gravity falls. In this experiment, flesh flies with halteres removed were unable to respond to the fall with a body movement, as intact flies were (Fig. 2). In fruit flies, which do not oscillate their halteres while standing or walking, this was not the case: there was no effect of haltere removal on their behavior. This experiment suggested that some species of flies can use their halteres to sense gravity and respond to sudden falls (Daltorio and Fox, 2018).

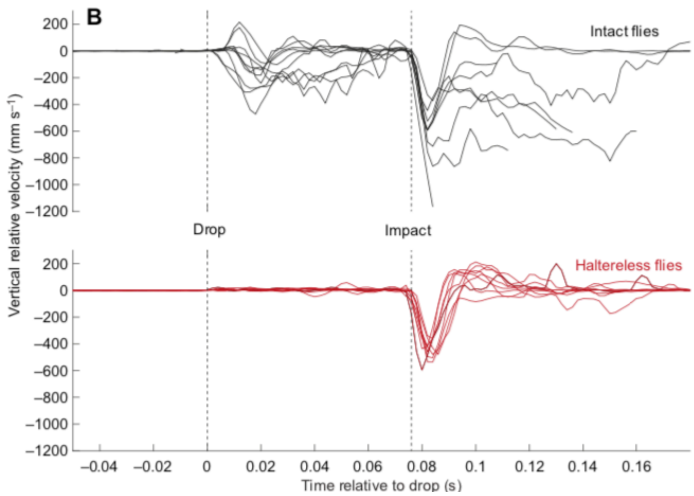

Figure 2. Intact flies experiencing a gravity fall will adjust their center of mass (top traces, time between drop and impact). Flies with halteres removed do not respond to the drop and only move their bodies following impact.

Takeoff: We used high-speed videography to film free takeoff behavior in several families of flies. We found that fly families in the Calyptratae clade (a large group that includes flesh flies, house flies, and blow flies) show faster takeoffs than other flies (Yarger et al., 2021). Removing the halteres of these flies made their takeoffs less stable and much slower, due to slower extensions of their jumping legs (Fig. 3). Haltere removal did not influence the takeoff speeds of other flies outside the Calyptratae. These results indicate that this group of flies may have expanded the use of their halteres to serve behaviors other than flight, perhaps improving their ability to avoid predation by stabilizing a rapid takeoff.

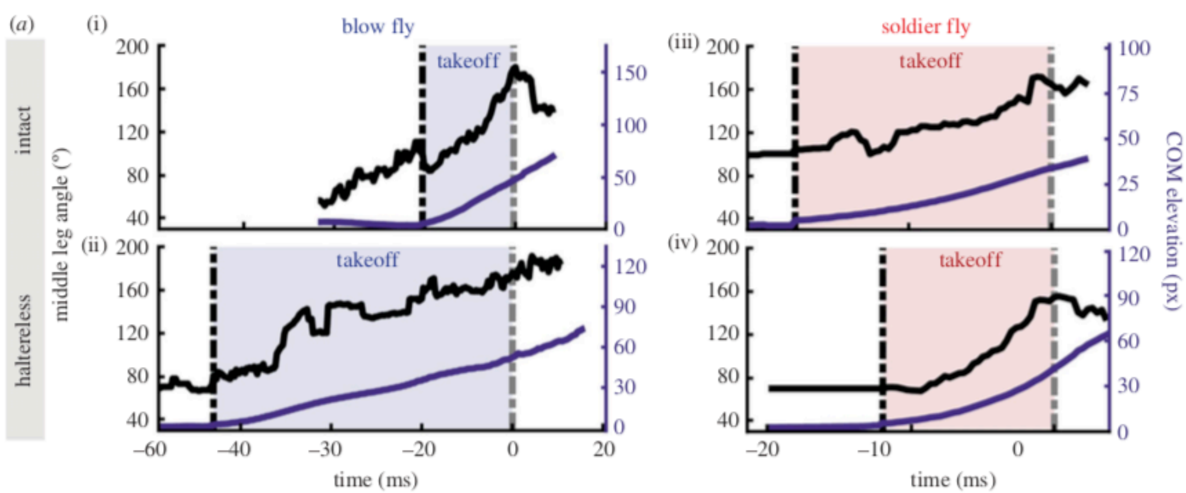

Figure 3. Example traces of takeoff behaviors of a fly in the Calyptratae (a blow fly; left, blue) and a fly outside of the Calyptratae (a soldier fly; right, red). Haltere removal has a significant slowing effect on the leg extension speed and overall takeoff speed of the blow fly but no effect on the soldier fly.

Outcomes of Aim 2

Our previous knowledge of haltere inputs to the nervous system was limited to anatomical descriptions of the targets of the primary afferents and to electrophysiological recordings of these target neurons, namely the wing-steering motoneuron mnb1 and the motoneurons of the neck. We recorded activity of the central complex of the brain, an integration area known to receive multiple sensory inputs and direct motor output, while we oscillated the halteres of quiescent flies (Kathman and Fox, 2019). In this experiment, the fly was quiescent and restrained in a plastic tube while we oscillated the haltere with a small motor. A strong magnet was glued to the motor, and a small iron filing was glued to the haltere such that the magnetic field would oscillate the haltere without any physical contact. Removing the magnet and
repeating the stimulus protocol provided a control for any physical or visual effects of the motor's oscillation unrelated to the haltere stimulation (Figure 4).

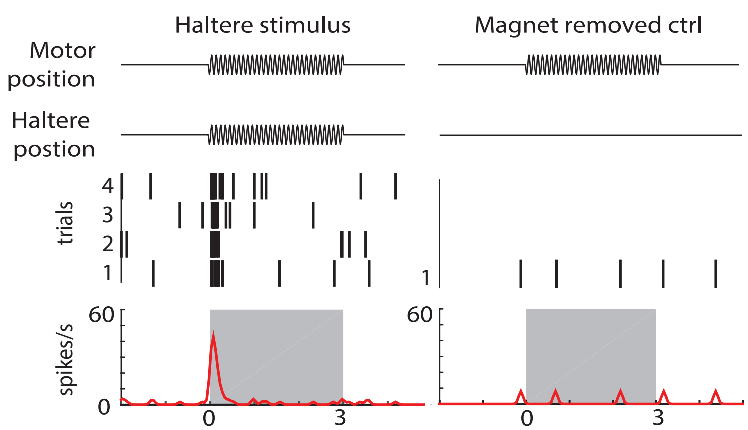

Figure 4. Peri-stimulus response of one unit to haltere oscillation trials (left column) and control trials (right column). The motor is oscillating at 60 Hz in this example, as represented by a schematic (top). A raster plot (middle) shows spike times from four trials of 60 Hz haltere oscillation and one trial of the control. An estimation of firing rate (bottom) from all trials (only one trial of control) shows the average unit response during the stimulus epoch (gray box).

First, we noted that in some units in the central complex, oscillations of the haltere resulted in spiking activity that was phase-locked to the timing of the haltere oscillation (Figure 5). This is similar to the mechanism of encoding used by the primary afferent neurons of the haltere, which precisely and rapidly fire spikes at the same point in each cycle of the haltere's oscillation. However, we measured the spike-timing precision in units of the central complex and found that is it not accurate enough for this to be the most likely mechanism of information transmission.

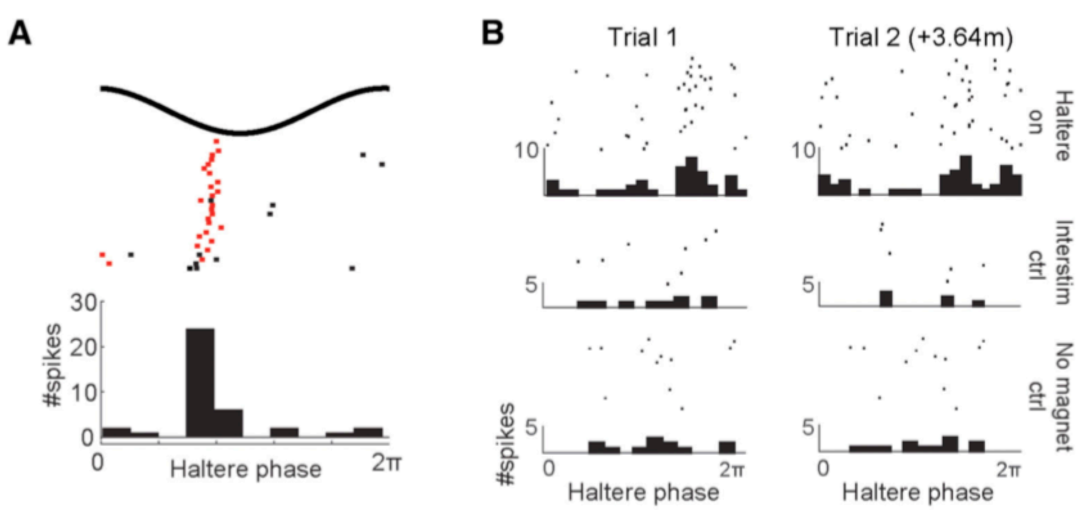

Figure 5. A) Haltere oscillations (top) are repeated and response times are plotted in red (for the first spike in the oscillation) or black (subsequent spikes). A histogram of the spike times (bottom) shows a preferred firing phase. B) This preferred firing phase persists across trials but is not observed during period with no stimulation or during control experiments with no magnet.

Instead, units of the central complex change their firing rate upon initiation of haltere oscillations. Haltere-responsive units fell into two major response classes: some units showed firing rates that were linearly related to the haltere's oscillation frequency (Fig. 6, left), and others increased their firing rate when the haltere was oscillated, but this increase was unrelated to the frequency (Fig. 3, right). This difference in response properties suggests that CX neurons may be encoding multiple types of information: linearly encoding units may inform the brain about specific aspects of haltere motion, while units that show a general excitation by haltere motion may inform the brain of the fly's behavioral state.

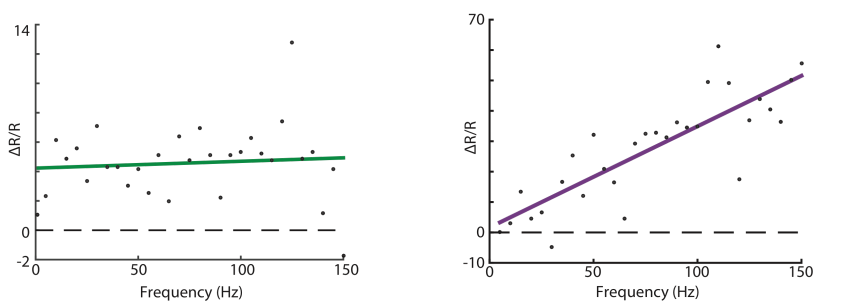

Figure 6. Haltere oscillation rate vs. increase in firing rate over baseline for two representative CX units. The unit shown at right is generally excited by haltere input but does not encode its frequency, and the unit shown at left linearly increases its firing rate with increasing haltere oscillation frequency.

Significant findings pursuant to Aim 3

In Aim 3, we determined how neurons of the CX respond to multimodal stimulation from both the halteres and the visual system. We added an LED arena to our experimental rig and provided wide-field stimuli in multiple directions and at multiple speeds. We found that some units changed their responses to visual stimuli when the halteres were moving. For example, the unit shown in Fig. 7 does not show strong responses to visual stimuli when the haltere is stationary. When the haltere is oscillated at a frequency above 10 Hz, however, the unit responds preferentially to forward thrust. This multimodal integration suggests that CX neurons can modulate their responses to both stimuli in a complex and possibly state-dependent way. Our recordings of CX neurons in behaving flies will further elucidate the interactions between vision, haltere movement, and behavior.

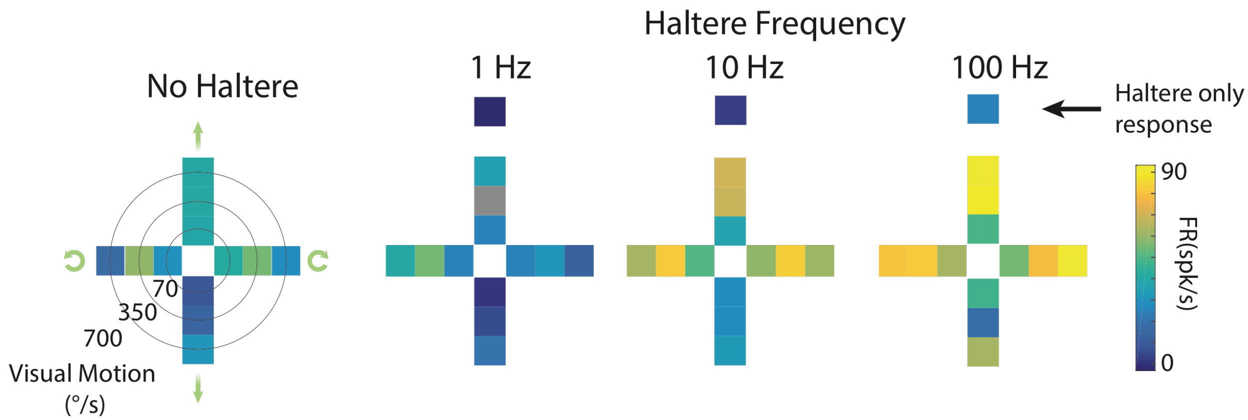

Figure 7. Firing rate of a central complex neuron during combined haltere and visual stimulation. **Left:** Responses to stimulation with wide-field visual motion at various directions (vertical axis: thrust, horizontal axis: yaw) and speeds. In the absence of haltere input, this unit is not selective for a specific visual direction or speed. **Middle to right:** When the haltere is oscillating, this unit shows selectivity for forward thrust at higher speeds. Spike rates are higher when the haltere is moved at a higher frequency, regardless of visual stimulus. This neuron is an example; we found a diversity of responses among neurons in the central complex.

Currently, we are recording activity from the central complex of flies as they walk on an air-supported Styrofoam ball. Several experiments have been performed with successful recordings, and analysis is currently underway. Additionally, Dr. Jeremy Didion, a postdoc supported on the YIP award and now on our new AFOSR grant, has refined the post-experiment anatomical verifications to allow us a better picture of where the brain recordings are taking place (Fig. 8). Because these recordings are performed extracellularly, the neural probes are dipped in a dye (DiI) before the experiment begins and brains are sectioned after the experiment and imaged with a confocal microscope to determine the precise location of the recording. Counterstaining the brain with DAPI, which labels DNA and thus can allow cellular-level resolution, improved the image of the brain and allowed us to better observe the subdivisions of the central complex. Dr. Didion's improved technique will provide a better understanding of which units are performing which tasks in the brain.

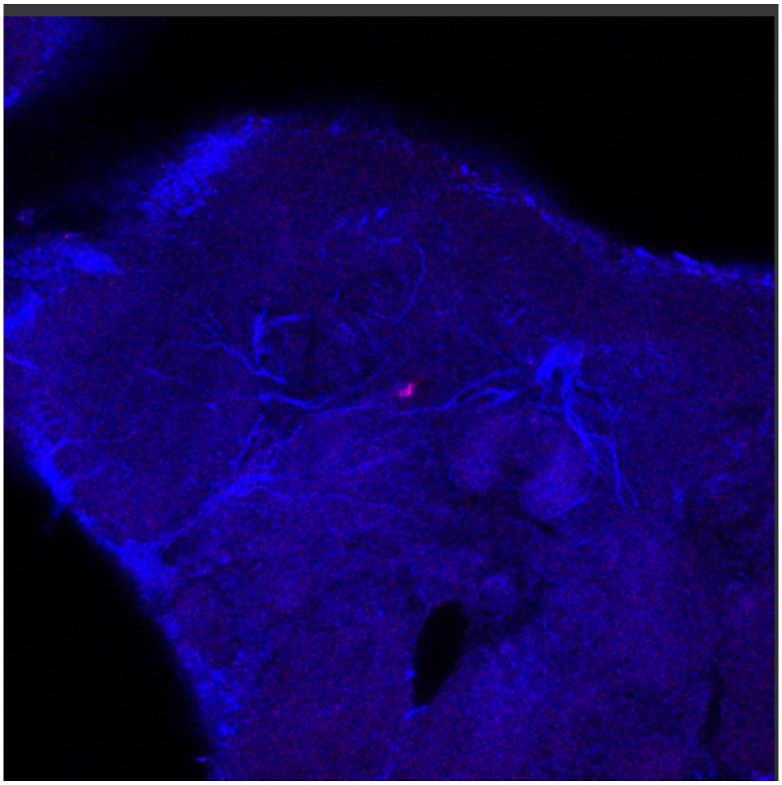

Figure 8. Section of the brain of a flesh fly (Sarcophaga bullata) showing the location of an injection of DiI indicator (pink). Using DAPI staining, we were able to improve the resolution of our neuroanatomy images for a better picture of the location of the probes in the brain.

Future directions

This grant spurred research ideas that led to a recently-funded AFOSR grant. We will measure and model the forces on the haltere to examine how sensory input changes when flies experience various perturbations, including standing falls, swinging falls, and wind gusts. We will also continue to record neural activity in the central complex during walking and flying behavior, comparing the results to the experiments in quiescent flies that were published during this grant.

Publications and presentations resulting from this funding

This grant resulted in eleven peer-reviewed publications, thirteen published abstracts, and thirteen invited talks from the PI. The postdoc funded by this project (Nick Kathman) has moved on to a second postdoctoral position at NYU in Dr. Kathy Nagel's lab. His work on multi-modal integration in the central complex has formed a basis for his work there as he studies the neural mechanisms of olfaction and antennal wind-sensing in fruit flies. A graduate student who contributed to the project (Alex Yarger) is now a postdoc in Huai-Ti Lin's lab at Imperial College London, where she has expanded her study of haltere mechanosensors to electrophysiological recordings of the mechanosensors on dragonfly wings. She expects that the skills she learned while working on some of the aims of the YIP award will help her to understand how mechanosensation enables rapid flight in diverse animals.

Relationship between this YIP award and the AFOSR Center of Excellence for Nature-Inspired Flight Technologies and Ideas

The YIP award supported significant, ground-breaking research within the Fox lab. In the duration of this funding, the lab was also partially supported by the AFOSR Center of Excellence for Nature-Inspired Flight Technologies and Ideas (NIFTI), a multi-university collaboration. This award provided significant opportunities for the personnel supported by the YIP award to present research and form national and international collaborations. The CoE spurred a collaboration between the Fox lab and the lab of Tom Daniel at the University of Washington that has currently resulted in two published abstracts and one publication (Mohren et al., 2019). Work from this collaboration continues on in our currently funded AFOSR grant. During her time as a graduate student in the Fox lab, Dr. Alex Yarger was a recipient of an AFOSR International Supplemental Student Exchange Program through NIFTI, and her travels to Imperial College London established a pathway to her postdoctoral position there. Dr. Michael Rauscher, a graduate student who participated in YIP and NIFTI projects, established a collaboration with Dr. Gabriella Wolff (then a postdoctoral associate with Dr. Jeff Riffell, now an assistant professor at CWRU) that led to a postdoctoral position that will begin in September 2021.

Conclusions and perspective

The work performed during the YIP funding period significantly expanded our understanding of haltere input to the fly brain. We showed that halteres are used for several behaviors in addition to flight, and we demonstrated that haltere input reaches the central brain and is integrated with vision. These projects have led to new areas of research to understand how the fly might parse information coming from the halteres during different behaviors, and how mechanosensation can be combined with vision to lead to robust, flexible takeoff and flight.

Daltorio, K.A., Fox, J.L., 2018. Haltere removal alters responses to gravity in standing flies. J. Exp. Biol. 221. https://doi.org/10.1242/jeb.181719

Kathman, N.D., Fox, J.L., 2019. Representation of haltere oscillations and integration with visual inputs in the fly central complex. J. Neurosci. 39, 4100–4112.

Mohren, T.L., Daniel, T.L., Eberle, A.L., Reinhall, P.G., Fox, J.L., 2019. Coriolis and centrifugal forces drive haltere deformations and influence spike timing. J. R. Soc. Interface 16, 20190035–20190035. https://doi.org/10.1098/rsif.2019.0035

Mureli, S., Thanigaivelan, I., Schaffer, M.L., Fox, J.L., 2017. Cross-modal influence of mechanosensory input on gaze responses to visual motion in Drosophila. J. Exp. Biol. 220, 2218–2227. https://doi.org/10.1242/jeb.146282

Rauscher, M.J., Fox, J.L., 2021. Haltere and visual inputs sum linearly to predict wing (but not gaze) motor output in tethered flying Drosophila. Proc. R. Soc. B Biol. Sci. 288, 20202374. https://doi.org/10.1098/rspb.2020.2374

Yarger, A.M., Fox, J.L., 2016. Dipteran Halteres: Perspectives on function and integration for a unique sensory organ. Integr. Comp. Biol. 56, 865–876. https://doi.org/10.1093/icb/icw086

Yarger, A.M., Jordan, K.A., Smith, A.J., Fox, J.L., 2021. Takeoff diversity in Diptera. Proc. R. Soc. B Biol. Sci. 288, 20202375. https://doi.org/10.1098/rspb.2020.2375