Reverse Engineering Animal Vision with Virtual Reality and Genetics

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Neuroscientists are using virtual reality systems, combined with other advances such as new molecular genetic tools and brain-recording technologies, to reveal how neuronal circuits process and act on visual information.

One major goal of modern neuroscience is to reverse engineer the brain and build an understanding not just of what pieces are important and how they are connected, but also how they work, ultimately at the biophysical level. Today, virtual reality (VR) experiments—complemented by advances in diverse fields such as genetics, brain-recording technology, and data analysis techniques—are revealing new insights into the mechanisms by which animals see.

What algorithms does a fruit fly use to regulate its flight altitude?[1] How does the brain of a zebrafish learn its own strength when swimming?[2] How do neurons in the mouse hippocampus encode the animal's position in a cognitive map?[3] These are the types of questions neuroscientists have addressed in the past few years using VR.

A fundamental aspect of biological vision is that it is used to guide locomotion, which in turn influences subsequent visual input. This nontrivial point creates substantial experimental requirements for a scientist wishing to systematically investigate vision. In particular, it requires the ability to manipulate sensory-motor feedback. Indeed, a major reason for the importance of VR within neuroscience is that it allows the experimenter to alter feedback in precisely defined ways. In doing so, vision scientists can ask a wide range of questions about visual processing, visual memory, and visual navigation. Furthermore, understanding the anatomy and physiology of neuronal circuits that implement such computations has become

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Fly Genetics for Neurobiology

Drosophila melanogaster—the fruit fly—is a favorite research subject among neurobiologists due to powerful genetic tools now available. A key genetic technology, the GAL4-UAS system, allows a modular specification of what cells drive the production of proteins of interest.

A *GAL4* line is a strain of flies that have DNA coding for the yeast protein GAL4, which is integrated somewhere in their genome. The location of this *GAL4* sequence insertion into the fly genome governs which cells produce GAL4 protein. A *UAS* line is a strain of flies containing the upstream activating sequence (*UAS*), also from yeast, and following this *UAS* is more DNA coding for a chosen protein of interest. The *UAS* is bound by the GAL4 protein and initiates the process that leads to production of the protein of interest.

When a GAL4 fly and UAS fly are mated, some offspring will contain both genetic components and thus will produce the target protein encoded by the UAS parents in the cells specified in the GAL4 parents. To visualize the location of GAL4-driven expression, we use this technique to drive production of green fluorescent protein by crossbreeding each of several *GAL4* lines with a *UAS-GFP* line, as Figure A shows. By imaging the resulting green fluorescent protein (GFP) expression under a microscope, we learn in which cells the *GAL4* line is active.

In addition to such anatomical experiments with *UAS-GFP*, other designer proteins can be introduced for other purposes. Toxins can interfere with cellular processes such as neurotransmission or even trigger cell death. Light- or heat-activated ion channels can cause remote-control activation or inactivation of neurons. Fluorescent proteins that change intensity or color based on neuronal activity can be used to record ongoing actions of the brain. To perform such experiments, we cross the original *GAL4* line with a *UAS* line carrying the appropriate protein coding sequence. Given the rapid life cycle of flies, this modular system allows the creation of flies with the desired properties in a matter of weeks.

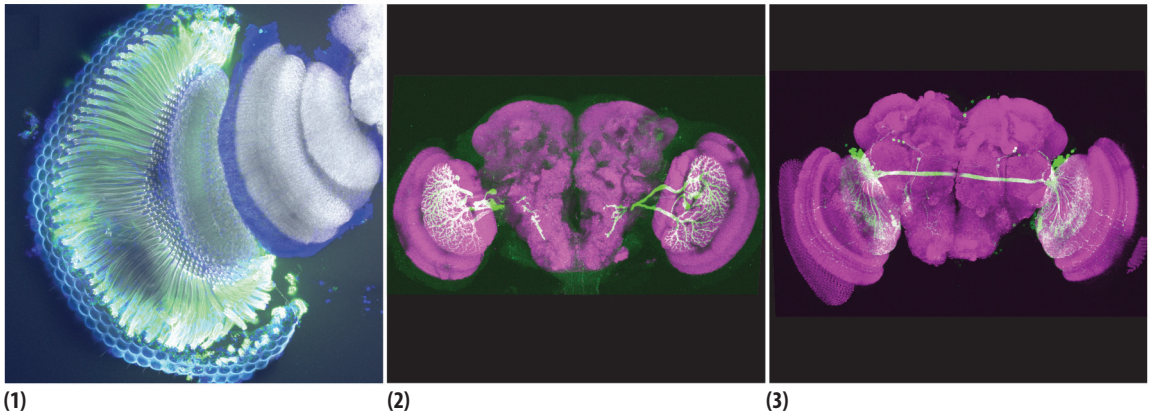


Figure A. Genetic access to individual cell types of the fruit fly brain. (1) The optic lobes of the fly showing "neurocrystalline" photoreceptors in green, cell bodies in blue, and all neural connections in grey. (2) The entire fly brain in purple, with neurons called the HS (horizontal system) cells in green through the use of an HS-specific *GAL4* line (GMR81G07-GAL4) and *UAS-GFP*. (3) DCN (dorsal columnar neurons) cells in green (using ato-Gal4-14a-GAL4 and UAS-GFP).

a particularly hot topic in recent years. VR is playing a key role in many laboratories engaging in these diverse streams of work.

Often, biologists concentrate on understanding particular animal species because such model organisms have properties favorable for the phenomenon under study. *Drosophila melanogaster*—the fruit fly—is one such example for those scientists wishing to understand visual processing. Under the lattice of the compound eye is brain tissue so precisely and repetitively organized that it may be described as a "neurocrystalline" structure. Furthermore, the fly eye was an early testbed for geneticists and molecular biologists to understand fundamental principles of development, and today the power of molecular genetic tools enable an understanding of vision in ways that were not possible before, as illustrated by the sidebar "Fly Genetics for Neurobiology."

To understand *Drosophila*'s visual system, we have developed an open source VR framework called FlyVR (www.flyvr.org). We have also applied this framework to the visual system of the large hunting spider *Cupiennius salei* and are beginning to collaborate with other labs on other species.

ANIMAL NEUROSCIENCE EXPERIMENTS

In flies and other animals, one powerful approach to clarify brain structure and function is a well-controlled genetic experiment. Two strains of an animal are bred to have identical genetic information with one exception—DNA that specifies, for example, killing or silencing specific

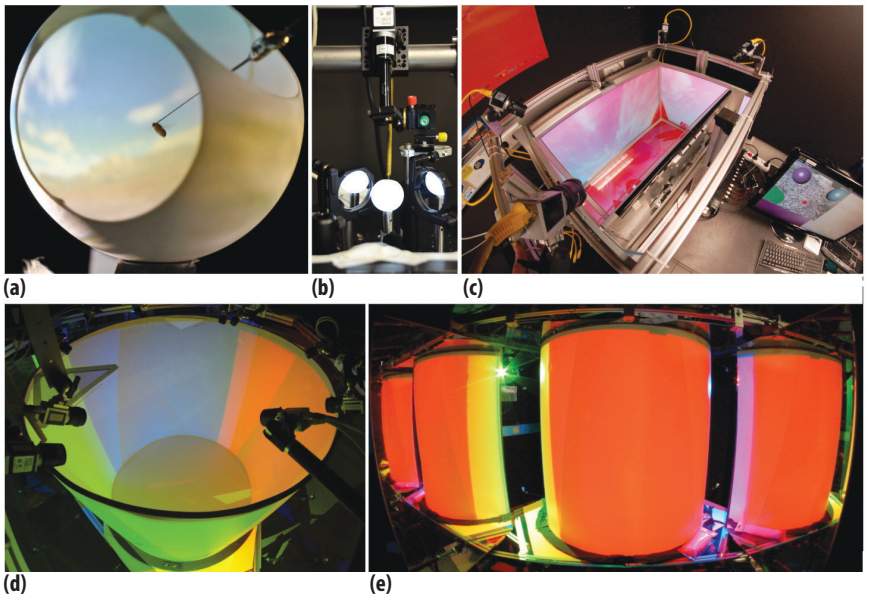


Figure 1. Examples of virtual reality implementations using FlyVR. (a) A fruit fly is rigidly tethered at the center of a spherical projection screen. (b) The computer-generated scenery is projected onto the outside of the sphere using a 120-Hz projector and two mirrors to achieve panoramic coverage. (c) The FlyCube, a rectangular VR arena using five 120-Hz LCD monitors, tracks freely flying flies using multiple cameras operating at 120 Hz. Subjects are tracked under illumination from red light using the monitors' red channel, and visual stimuli are displayed using the blue and green channels. (d) The FlyCave is a 1-m diameter, 1-m high cylindrical panoramic VR arena. Flies are enclosed inside the cylinder and tracked under infrared light using 12 cameras operating at 100 Hz. (e) The computer-generated scenery is projected onto the outside of the cylinder using three 120-Hz projectors and six mirrors.

neurons. Within a given assay, comparing performance of the two genotypes will consequently reveal the effect of the particular neurons and thus illuminate their role. While this is a conceptually simple approach, a significant challenge is that variability in responses among individual animals, or even from trial to trial, can be significant. This is especially true if the readout is some aspect of behavior, which is notoriously variable. Performing many experiments is one way to deal with the problem, and thus a desirable characteristic for an animal-behavior VR system is to be automated and efficient.

Another powerful approach commonly used in neurobiology is to record the activity of neurons during visual stimulus presentation, potentially simultaneously with performance of a behavioral task. Combined with genetic manipulations and VR experiments, these brain recordings are extremely powerful. Nonetheless, such experiments might fail to reveal mechanisms that evolved in natural conditions because obtaining such delicate recordings typically involves rigid immobilization of the animal at the focus of a microscope. This rigid tethering dramatically alters sensory feedback to the animal, especially mechanical sensations.

Consequently, in our work, we seek to achieve high throughput and investigate naturalistic behavior by allowing animals to move freely, and we utilize genetic techniques to introduce controlled differences between strains. Figure 1 shows some example FlyVR implementations.

ANIMAL-BEHAVIOR VR SYSTEM REQUIREMENTS

Given the experimental approaches described above, what are the requirements for an animal-behavior VR system?

First, neuroscientists want to specify spatial, luminance, and chromatic properties across an animal's entire visual field with high accuracy. Model organisms such as flies, mice, and zebrafish have very large fields of view, so implementing an appropriate display is challenging.

Second, to appropriately alter sensory feedback, total system latency should be low. Latencies of 100 milliseconds are sufficient for some types of experiments, but latencies around 16 ms are required for human perceptual stability. Given that many animals have faster visual and motor systems than humans, some experiments could place extreme demands on system latency.

Third, neuroscientists perform experiments in very different conditions. Animals could be fixed at the focus of a microscope or free to move, so an implementation supporting different display geometries and technologies is necessary. Flat display panels, curved screens, and multiprojector systems should all be supported.

Finally, many neuroscientists are not computer programmers, and those who are often lack expertise in specialized fields such a graphics and tracking. Thus, an animal-behavior VR system must be usable by nonprogrammers.

NATURAL VS. ARTIFICIAL STIMULI

Most visual neuroscience experiments employ highly artificial stimuli such as sinusoidal grating patterns or checkerboards. Historically, this was partly due to technical limitations in stimulus generation, but such simple stimuli remain useful because they give the experimenter precise ways to alter the stimulus and quantify

the response. However, with advances in display technology and computer power, the past decade has been rich with efforts to extend sights to less constrained and more natural conditions.

Nevertheless, to reconcile new and old results and to utilize powerful system identification methods, many experiments will continue to employ simple stimuli whereas others will probe new behaviors using more photorealistic stimuli. However, the same ultimate goal remains—to understand how the animal operates under a wide range of conditions, particularly those under which evolution shaped the animal being studied.

IMPLEMENTING VR FOR ANIMAL BEHAVIOR

How do the requirements for animal neuroscience translate into actual VR system implementation? In particular, what are the constraints and features of such a system compared to more conventional VR systems for human use?

While many model organisms have larger fields of view than humans, their spatial resolution is usually worse. The horizontal visual acuity of *Drosophila*, zebrafish larvae, and *Cupiennius* is about 5°, 3°, and 1°, respectively. Thus, the VR resolution requirements of animals can be less than those required of humans (0.008°). Other aspects can also be different than human vision. *Drosophila*, for example, cannot see red, are most sensitive to green, and are sensitive to polarization of light in some parts of the retina. Many model organisms have little or no binocular overlap and closely spaced eyes, leaving little room for stereo disparity as a visual cue.

Due to the biological and experimental constraints of the animals under test—such as their physical size, visual field, preparation method, and visual sensitivity—the display configuration's geometry, luminance, and resolution properties are all interdependent.

FlyVR

FlyVR supports diverse display hardware and geometric arrangements, both naturalistic and artificial visual stimuli, and multiple real-time tracking systems. While several existing VR frameworks have implemented various aspects of our system requirements, we are not aware of any that would meet all of them. Most such frameworks are optimized for human VR needs[4] and thus often support features not required for animal-behavior VR including sophisticated interaction with virtual objects in the scene, menu or GUI elements, head-mounted displays, and haptic feedback devices. Other animal-behavior VR frameworks do not support an observer that translates relative to the display surface[5] or are limited to special configurations.[6] FlyVR allows flexible specification of geometry and luminance and avoids limiting the display geometry to common constructions like computer-assisted virtual environments (CAVEs),[7] single-planar monitors, multiprojector planar surfaces, or low-resolution LED panels.[5] Furthermore, the system supports both tethered and freely moving animals.

To achieve the required flexibility in display hardware, FlyVR isolates the geometric and luminance calibrations from the experiment being performed and the system's display configuration, as Figure 2 shows. For example, in our VR environments using projectors, the beam paths from the projectors in general overlap one another on the projection surface. FlyVR uses blending masks that define per-pixel attenuation to ensure that the luminance of a given patch of screen is independent of how many different beam paths illuminate it.

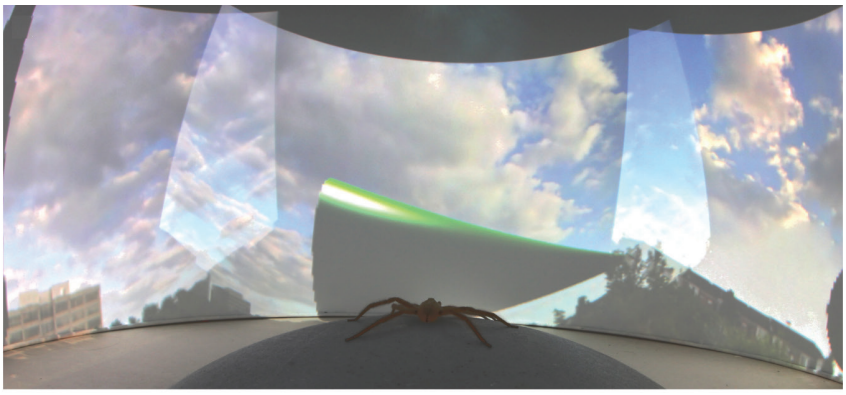
While automatic calibration systems are popular in human VR (using the tracking system to observe the projectors directly), this approach is not always possible with animals. In our experience, this is due to either scale issues—physically small display surfaces, such as that in Figure 1a, combined with low-resolution projectors—or the tracking system's need to be insensitive to computer-generated stimuli. To support these use cases, FlyVR enables semiautomatic calibration approaches in which the experimenter manually marks key points on the display surface.

TRACKING TECHNOLOGY

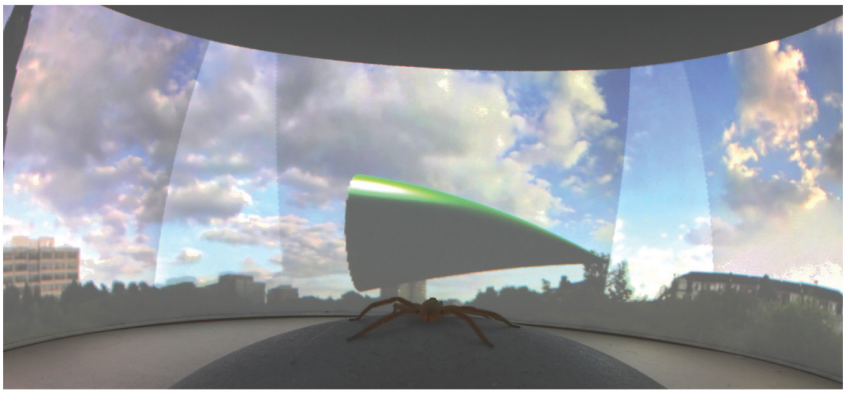
Manipulation of an animal's sensory-motor feedback loop requires tracking the animal's actual or intended movements and using this to drive the VR. This is often done using computer vision techniques. A commonly employed method is to use infrared light with spectral-blocking filters to eliminate the visible wavelengths generated by the computer displays from reaching the cameras and thereby causing spurious tracking.

For rigidly fixed animals, the pose in the virtual environment must be artificially generated by a physics model that takes measured motor output from the animal and simulates its motion. For our work on tethered flies, we immobilize the fly by gluing its thorax and head to a small metal rod (Figure 1a), and estimate turning torque by measuring wing-beat amplitude using computer vision, as Figure 3a shows. For freely flying flies, we measure position directly. To avoid the need to mount retroreflectors, markers, or LEDs on small flies, we use a low-latency multicamera markerless tracking system,[8] as shown in Figure 3c. Intermediate configurations, such as the spider on a treadmill in Figure 3b or a restrained mouse with head-movement freedom, allow the animal some degree of motion while limiting overall movement outside the tracking region.

Total VR system latency comes from three major components: tracking, rendering, and display. While measuring latency is fairly straightforward for any of these components individually or as a complete system, it is less clear how to measure what latency is sufficient to perform a particular



(a)



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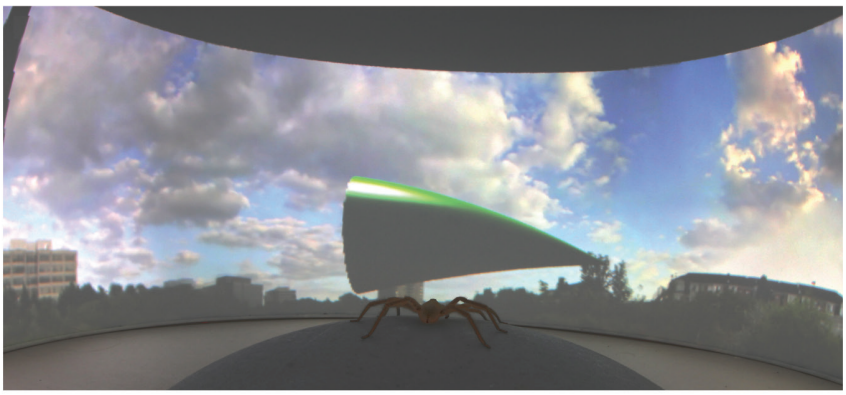

(c)

Figure 2. Geometric and luminance correction in a VR cylinder for spiders. (a) Original projector views. (b) Geometry corrected but without luminance correction. (c) With luminance blending enabled. The spider walks freely on a 0.8-m-diameter air-supported trackball.

experiment. With human subjects, it is easy to ask about the experience, but this is not possible for nonverbal animals. Furthermore, there are latencies associated with conventional cameras and computer displays that would be difficult or expensive to overcome. In particular, the latencies due to transferring image data from cameras and frame data to the displays dominate total FlyVR latency, which is between 50 and 80 ms depending on the exact configuration. Artificially adding a small amount of additional latency does not reveal substantial changes in the behavioral results we measure, and thus we believe that removing additional latency would also result in little change.

CASE STUDY: ACTIVE CONTROL OF *DROSOPHILA* FLIGHT

FlyVR system performance can be demonstrated with an experimental protocol we developed to study *Drosophila* whereby we actively direct them to fly along an arbitrary preprogrammed trajectory, as Figure 4 illustrates. This procedure takes advantage of the fly's robust visual reflexes as well as our ability to track such a fly flying freely and update visual stimuli in real time. A high-level experimental controller combines these aspects together.

To maintain stability in the face of external perturbations, such as a gust of wind, most visual animals possess an innate response to wide-field visual motion. In flies, this is known as the optomotor response.[9] When flies like *Drosophila* are presented with a rotating stimulus, they turn with the stimulus to eliminate the unintended motion. Humans encounter a similar feeling when seated in a stationary train and a neighboring train begins to move.

Our control procedure updates the rotational velocity of the visual surroundings to cause the fly to steer in the direction of our choosing. With this procedure, we can direct the fly around a looping path and thus gather large quantities of data on an individual fly's visuomotor performance. Because the procedure is completely automated, we can do this repeatedly and gather hundreds of trajectories in several hours. We find, for example, that blocking

one set of neurons requires the visual stimuli to be faster to confine the flies to the predefined path. We are following up on such results to discover the impairment's exact nature and how connected neurons are involved.

Due to the extensive body of literature on fly visual reflexes in which the experiments were done on rigidly fixed animals, another aspect of our work is to characterize free flight and reconcile results from the two conditions. Furthermore, because FlyVR is capable of producing similar visual input to both tethered and freely flying animals, it presents an ideal opportunity to study effects of mechanosensory disruption and to understand the ways in which the act of fixing an animal, essential for many kinds of brain recording, alters behavioral responses.

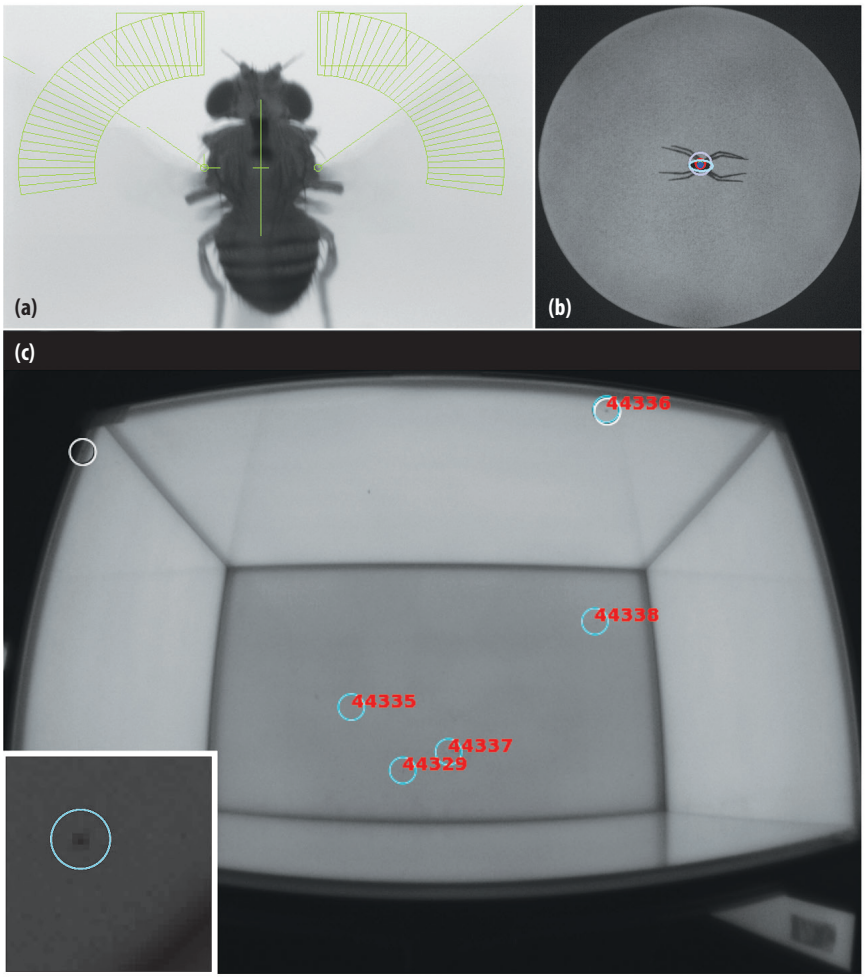

Figure 3. Real-time animal tracking. (a) For rigidly fixed animals such as this fly, body movements or neural recordings can be used as input to a physics simulation of observer pose. Here, the lines indicate wing-beat amplitude—a proxy for torque. (b) For loosely tethered configurations, such as this spider on a spherical treadmill, the animal is generally confined to a small region but the movement dynamics can be at least partly observed directly. (c) For freely moving animals, pose is directly measured and no physical simulation is necessary. In this case, we track multiple flies simultaneously but configure FlyVR to "lock on" to a single fly once it reaches an experiment trigger condition such as location within a particular subvolume—thus, only one fly is in VR at any instant.

CASE STUDY: VISUALLY GUIDED BEHAVIOR IN *CUPIENNIUS*

Cupiennius has long been a focus of research for its well-developed sensory abilities. In our setup, the spiders walk freely on a 0.6-m infrared illuminated polystyrene sphere (Figure 2). The sphere is actively driven by two sets of motors to compensate spider locomotion, resulting in a quasi-stationary but unrestricted walking animal.

We have been able to show that *Cupiennius* moves toward large black bars, likely to seek dwelling plants. It discriminates between different, simultaneously presented objects—for instance, preferring upright bars to oblique ones.[10] This behavior can be used to test which properties a spider relies on to choose a target. In another experiment, we test whether a spider uses objects' chromatic properties to detect targets. We introduce the spider to a virtual environment and prompt it to detect or discriminate among objects of different colors.

The novel combination of FlyVR with a locomotion compensator enables us to conduct larger numbers of successive experiments on an individual spider without extensive handling, thereby reducing stress-induced experimental artifacts. The VR setup allows us to create any desirable environment and to alter the visual complexity as well as properties of objects such as luminance and color, shape, size, position, distance, and motion.

FlyVR lets us unravel the naturally occurring visual parameters that *Cupiennius* might use in different visually guided contexts, such as searching for prey,[11] avoiding predators, and orientating toward dwelling

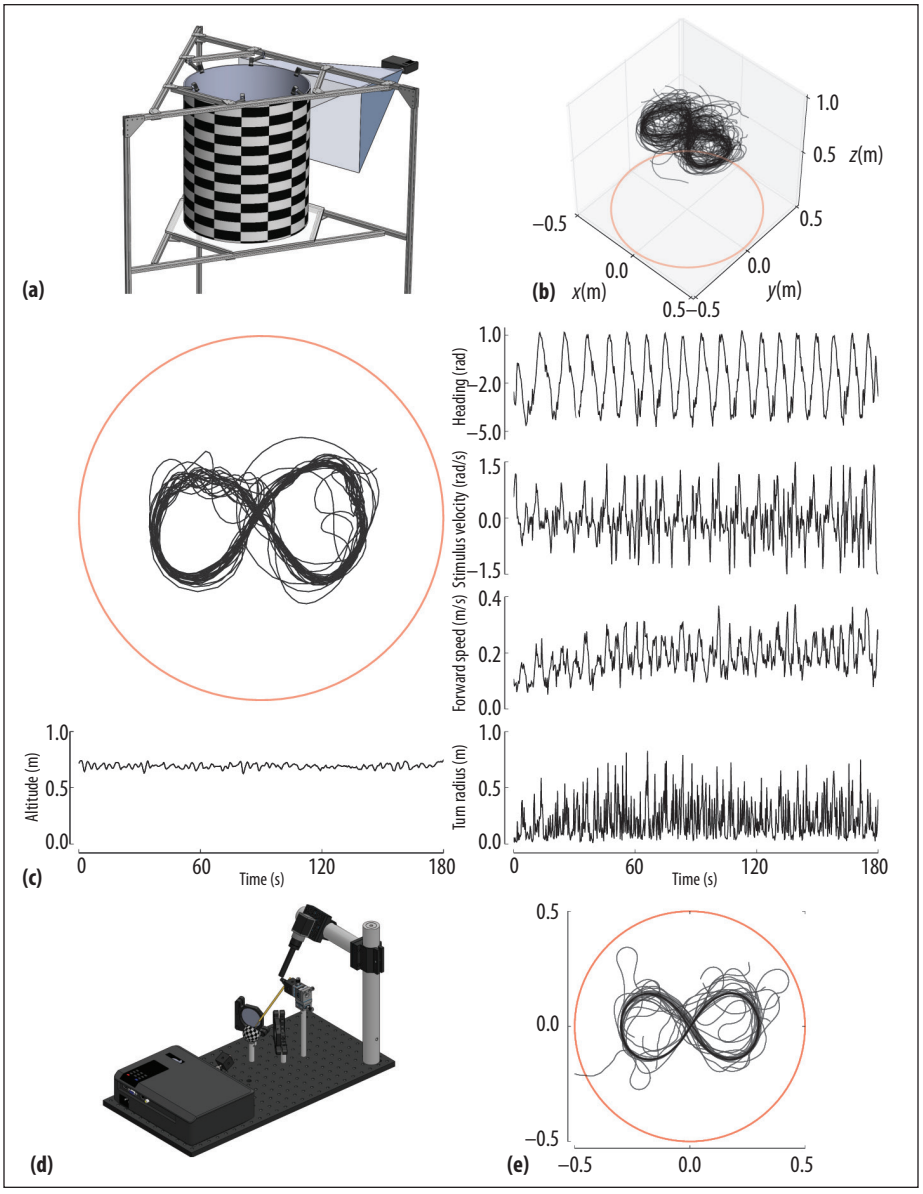

Figure 4. Active control of *Drosophila* flight. (a) The FlyCave arena. (b) Flight trajectories of multiple freely flying flies when engaged in an experimental paradigm that steers them to follow a figure-8 path by rotating their surroundings as a function of their current position and velocity. (c) Top view of a single long trajectory in the free-flight arena and multiple types of data collected. (d) A tethered-fly flight arena. (e) An individual flight trajectory of a tethered fly controlling a "flight simulator" based on tracking the fly's wing movements. Performing similar experiments in free flight allows us to rapidly perform many naturalistic experiments, whereas tethered configurations enable us to perform more precise measurements.

plants. Our work's ultimate aim is to understand how these parameters together influence visual decision making in spiders.

VR is a powerful technology for linking animal behavior with neuronal function, and a growing number of neuroscience laboratories are implementing VR in tracking and display systems. Together with other advances in molecular genetic tools, brain-recording techniques, and data analysis software, VR is allowing scientists to make substantial discoveries about how the brain works to control behavior in natural circumstances. C

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