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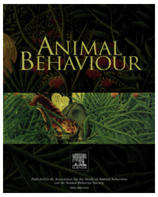

A virtual reality paradigm for the study of visually mediated behaviour and cognition in spiders

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Jumping spiders (Salticidae) are well known for their unique, high-acuity visual system and complex, visually mediated behaviour. To overcome the limitations of video playback and other open loop systems that are currently available for the study of visually mediated behaviour in jumping spiders, we developed a closed-loop, virtual reality (VR) system in which a spider on a spherical treadmill walks through a projected 3D world that updates in real time in response to its movements. To investigate VR as an experimental technique for spiders as well as validate it as a proxy of the real world, we conducted two experiments to assess whether individual behavioural tendencies and learning transferred from real to virtual environments. In the first experiment, we examined transference of individual behaviour tendencies (spontaneous locomotion and dark/light preference) between real and VR environments, and found that individual differences were conserved. In the second experiment, we investigated transference of beacon-learning tasks between real and VR environments. We found that spiders that had learned a beacon–nest site association in the real world tended to expresses similar associations in the virtual world. Virtual reality offers great promise as a new tool to explore the cognitive processes underlying vision-mediated learning, memory and navigation in jumping spiders.

Virtual reality (VR) is increasingly used in the behavioural sciences to overcome the constraints of traditional open-loop systems. In the context of animal behaviour, VR refers to a simulated environment that is sensed by the animal and is updated by the animal's actions (Dombeck & Reiser, 2012), giving an experience of immersion within the simulation (Sherman & Craig, 2002). VR, by design, is a closed-loop system, in which responses to virtual stimuli are tracked and used to update the next 'view' of the virtual environment in real time. Since virtual environments are typically implemented as automated, computer-controlled systems, virtual stimuli and world features can be designed to be 'photo-real', abstract, or even selectively modified versions of real-world stimuli. In this way, VR can provide a valuable bridge between ecological validity and experimental control, supporting rich, multisensory environments alongside precise control of experimental variables (Bohil, Alicea, & Biocca, 2011).

Understanding how 'simple' invertebrate systems handle the complexities of daily life can inform us about the cognitive processing requirements that are necessary and sufficient to accomplish a given task. Virtual environments, in various forms, have been utilized to dissect diverse aspects of invertebrate behaviour and physiology. Perhaps the most common form has been the flight simulator for tethered flying insects, where the subject's intention to turn in response to visual motion is measured with a torque meter or wing beat analyser and used to update a virtual environment in real time. Using this approach, researchers have studied optomotor responses (Bender & Dickinson, 2006), flight kinematics (Gray, Pawlowski, & Willis, 2002), navigation (Gotz, 1987; Reiser & Dickinson, 2010), visual learning (Dill, Wolf, & Heisenberg, 1993; Wolf et al., 1998), decision making (Brembs, 2011) and operant and classical conditioning (Brembs, 2000). Systems have also been designed for tethered walking invertebrates (Dahmen, 1980; Strauss, Schuster, & Götz, 1997; Takalo et al., 2012), and, recently, for subjects in free flight (Fry et al., 2004; Fry, Rohrseitz, Straw, & Dickinson, 2008).

Jumping spiders (Salticidae) are well known for their unique visual system and complex, visually mediated behaviour. In contrast to the compound eyes of insects, salticids possess four pairs of functionally specialized 'camera eyes', each of which uses a single lens to project an image on to the retina (Land, 2005). Their large forward-facing principal eyes feature colour vision (Land,

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1969b; Peaslee & Wilson, 1989), depth perception (Nagata et al., 2012), and a retina with spatial acuity that greatly exceeds that of any other animal with eyes of comparable size (Land & Nilsson, 2012). Smaller motion-detecting secondary eyes collectively enable the spider to view nearly 360^o of its surroundings (Land, 1971). By separating spatial acuity from motion detection, the modular design of the salticid visual system enables remarkable visual feats while minimizing costly increases in eye size (Land & Nilsson, 2012; Laughlin, de Ruyter van Steveninck, & Anderson, 1998). As a comparison, the high-resolution compound eyes of libellulid dragonflies combine these features in the same eye, but to achieve this their eyes are larger than the entire body of most salticids (Labhart & Nilsson, 1995).

Using optical cues alone, salticids can identify prospective mates and rivals, classes of prey and enemies, and features in their environment (Harland & Jackson, 2004; Jackson & Pollard, 1996; Tarsitano & Jackson, 1997). Vision is also central to intraspecific communication, such as during elaborate courtship displays (Elias, Land, Mason, & Hoy, 2006; Elias, Maddison, Peckmezian, Girard, & Mason, 2012) and during highly ritualized agonistic interactions between males (McGinley, Prenter, & Taylor, 2015; Taylor, Hasson, & Clark, 2001). Vision also plays a central role in cognition, with salticids relying on vision for complex decision making (Jackson & Cross, 2013), learning (Jakob, Skow, Haberman, & Plourde, 2007; Nakamura & Yamashita, 2000; Skow & Jakob, 2006), discrimination and categorization of prey (Dolev & Nelson, 2014), trial and error problem solving (Jackson & Nelson, 2011) and navigation (Hoefler & Jakob, 2006; Tarsitano & Jackson, 1997).

Salticids respond to video images of prey, enemies and conspecifics in a manner that closely resembles their responses to natural stimuli. Clark and Uetz (1990) were the first to exploit this capability, pioneering the use of video playback for studies of spider communication. Video playback proved a significant advance over previous methods, such as mirrors, 2D static images and dummy lures, and has served as a valuable tool over the past 25 years (e.g. Bednarski, Taylor, & Jakob, 2012; Harland & Jackson, 2002; Menda, Shamble, Nitzany, Golden, & Hoy, 2014). The researcher can control the timing and features of a visual stimulus while ensuring that an identical stimulus is presented across trials and test subjects (D'Eath, 1998). However, while video playback offers many advantages over the available alternatives, it also shares some of the same constraints that have limited the utility of previous methods (and see Fleishman & Endler, 2000; Fleishman, McClintock, D'Eath, Brainard, & Endler, 1998 for general limitations). In particular, video playback is an open-loop system in which sets of stimuli are presented independently of the subject's responses. This lack of contingency between cue and response, as well as the absence of normal visual feedback to a mobile subject, interferes with the perception of depth and motion and ultimately hinders the realism of the simulation (Woo & Rieucau, 2011; Zeil, 2000). A method that closes the feedback loop would offer significant advantages over traditional open-loop playback techniques.

The present study is the first to employ VR as an experimental approach to studying perception and cognition in salticids. In addition to detailing our VR methods, we validate VR as a representation of the real world (RW) through experiments demonstrating (1) consistency of individual differences across RW and VR contexts, and (2) transfer of associations learned in the RW to VR.

METHODS

Source, Maintenance and Preparation of Spiders

Mature female *Servaea incana* jumping spiders ($N = 56$) were collected from *Eucalyptus* trees in Sydney, Australia (Richardson & Gunter, 2012). Spiders were maintained in a controlled-environment laboratory (24–26 °C; 62–67% relative humidity; 11:1:11:1 light:dusk:dark:dawn starting at 0700 hours) where they were individually housed in cubic 1.125-litre plastic cages that had a 10 cm diameter mesh-covered opening on one side for ventilation. Each cage contained a folded sheet of paper (2 × 3 cm) that was shaped as a 'tent' under which spiders could shelter and build nests. Spiders were fed weekly on an alternating diet of two house flies, *Musca domestica*, or two Queensland fruit flies, *Bactrocera tryoni*. Supplementary moisture was provided by lightly misting each cage with a spray bottle once each week. No experiments were carried out during the first or last hour of the laboratory light phase.

In preparation for VR experiments, spiders were removed from their home cages and transferred in a 5 ml plastic vial to a refrigerator (4 °C) where they were cooled until quiescent (2–3 min), then placed on a chilled granite block. A 2 mm diameter neodymium magnet (0.4 mm thickness, 8.5 mg; approximately 13% of the average spider weight) was gently affixed to the dorsal carapace using a drop of dental cement (SynergyFlow A3.5/B3, Coltene Whaledent, Burgess Hill, U.K.), taking care not to cover the eyes. The dental cement was cured with blue light from an LED dental curing light source (SDI radii plus, Henry Schein Dental, www.henryschein.co.uk). Spiders were then returned to their cages and allowed to recover for a minimum of 24 h before being used in experiments. Spiders with magnets were maintained for 6 months following experimentation and we did not notice any changes in locomotion, behaviour or longevity in comparison to spiders without magnets.

At the start of each VR trial, spiders were gently lifted from their home cages using a magnetic pin and mounted so that they stood on a spherical treadmill (Fig. 1a). Movements of the treadmill were tracked and updated the virtual environment in real time (described below).

Following the completion of each VR trial, a strip of laminated paper was used to gently separate the neodymium magnet and magnetic pin, and each spider was returned to its home cage. Using prefixed magnets provided two significant advantages over direct tethering to a pin: (1) spiders could be tethered well in advance of a trial, and then have ample time to recover from stress associated with handling, and (2) each spider could be used repeatedly over days or weeks. The potential to use spiders repeatedly is valuable in this context since learning and memory trials typically occur over broader timescales than can be assessed within a single session.

VR System

A 3D-printed spherical treadmill was constructed using polylactide plastic. The treadmill comprised a semispherical chassis that held a 3.5 cm diameter, 230 mg expanded polystyrene ball, tracking sensors, and a clip above the ball to grip a magnetic pin that was attached to the magnet on the spider's carapace to hold the spider in place. The expanded polystyrene ball was supported by a constant, steady flow of air (Sparmax AT-250 mini-compressor; air flow meter, Comweld Medical, Preston, VA, Australia). The weight of the ball was unlikely to affect the spider's movement. The moment of inertia of the ball ($I_{\text{sphere}} = 2/5 \times mr^2$) was 112 700 mg mm^2, which was 70 times greater than that of the spiders' presumed moment of inertia (see Zurek & Nelson, 2012b for calculations); however, Land (1972) observed unimpeded movement in jumping spiders holding objects with moments of inertia 400 times greater than that of the spider. The spherical treadmill used here was similar to previously described optical mouse tracking systems (Harvey, Collman, Dombeck, & Tank, 2009; Takalo et al., 2012). Rotations of the ball were detected at a rate of 1000 frames/s using four PS/2 optical mouse (Logitech