

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



Tina Peckmezian\*, Phillip W. Taylor

Department of Biological Sciences, Macquarie University, Sydney, Australia

### ARTICLE INFO

#### Article history:

Received 23 March 2015  
 Initial acceptance 21 April 2015  
 Final acceptance 3 June 2015  
 Available online 13 July 2015  
 MS. number: 15-00234

#### Keywords:

jumping spider  
 learning  
 Salticidae  
 virtual reality  
 vision

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 express 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.

© 2015 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

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,

\* Correspondence: T. Peckmezian, Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia.

E-mail address: [tina.peckmezian@gmail.com](mailto:tina.peckmezian@gmail.com) (T. Peckmezian).

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° 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 (Hoeffler & 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, Shamblé, 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 & Rieucou, 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.

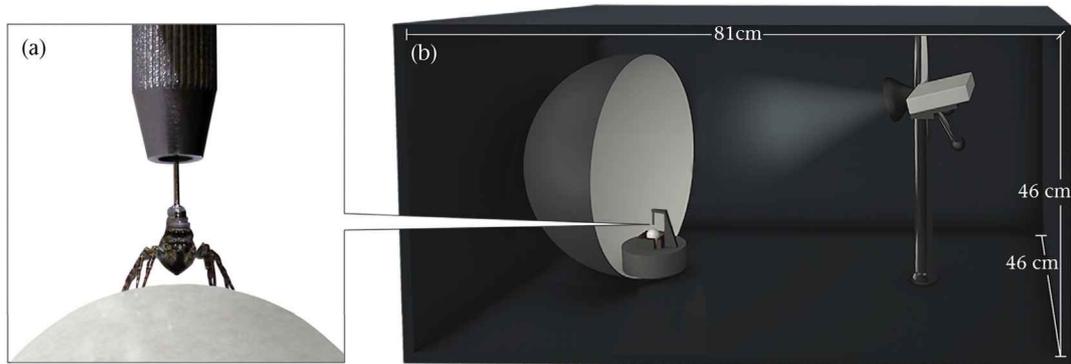
In preparation for VR experiments, spiders were removed from their home cages and transferred in a 5 ml plastic vial to a refrigerator (4 °C) where they were cooled until quiescent (2–3 min), then placed on a chilled granite block. A 2 mm diameter neodymium magnet (0.4 mm thickness, 8.5 mg; approximately 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](http://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<sup>2</sup>, 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



**Figure 1.** (a) A spider mounted above a spherical treadmill in preparation for a trial. A magnet is fixed to the spider's dorsal carapace and a magnetic pin holds the spider in position. (b) Schematic of VR system. Spiders are mounted above an air-supported spherical treadmill placed inside a hemispherical display screen. Dynamic computer-generated 3D environments are front-projected onto the screen via a pocket projector with add-on fish-eye lens, and spider movement updates the world in real-time (closed-loop).

International, Newark, CA, U.S.A.) sensors (ADNS 2610, Avago Technologies Ltd, Singapore) positioned along the midline, with opposing pairs averaged to reduce measurement noise. The sensors connected to an Arduino Uno microcontroller board, in which motion (translation in X, Y and Z as well as rotation about the yaw, pitch and roll axes) was read by custom-written software. Commands were sent from the microcontroller board to a computer in ASCII format via a USB cable connecting to a virtual serial communication port at a rate of 60 Hz (the closed-loop update rate).

The treadmill was positioned centrally in front of a hemispherical display screen (40 cm diameter, painted with flat white epoxy enamel). The virtual environment was projected onto the inner surface of the hemispherical display screen using an LED pocket projector (Qumi Q2, Vivitek, Hoofddorp, The Netherlands) with a supplementary fish-eye lens (Zeikos, Edison, NJ, U.S.A., 5.8 cm,  $0.43\times$  wide angle). For spherical, projector-based systems, a fish-eye design can produce higher image quality than traditional mirror-based systems (Takalo et al., 2012). This projector has a refresh rate of 60 Hz (at 1080p resolution) which is above the suggested flicker fusion frequency of salticid eyes (40 Hz, Forster, 1985). A planoconvex lens (2.5 cm diameter  $\times$  100 mm focal length, Edmund Optics, Barrington, NJ, U.S.A.) was used to focus the light from the projector into the fish-eye lens. The optics used here were chosen to maximize the 'fill' of the sphere while providing a focused image. A 3D-printed holder affixed the projector to its lenses, and this unit was mounted onto a movable arm approximately 25 cm above the base of the screen and 50 cm from the centre of the screen. The simulation projected  $160^\circ$  in both azimuth and elevation, covering the full field of view of both the forward-facing principal eyes and the anterior lateral secondary eyes, which have greater spatial acuity than the other laterally facing secondary eyes (Zurek & Nelson, 2012a). The entire system was enclosed in a light-tight black Plexiglas box to isolate spiders from distraction as well maximize projector brightness (maximum of 300 lumens in complete darkness; Fig. 1b).

Care was taken in adjusting the spider's position so that it could fully extend its legs and step normally, although jumping behaviour was inhibited by tethering. Most salticids, including *S. incana*, use stepping movements for normal locomotion in a manner similar to other spiders, and reserve jumping to span gaps, to escape enemies and for prey capture (Foelix, 2011). The inability to jump can be considered a limitation of tethered preparations in these circumstances, but is not expected to be a limitation under normal locomotion. Each spider was allowed 3 min to adjust to the set-up with the screen dark before the simulation was started.

### Visual Stimulus Design and Presentation

Three-dimensional simulated environments were created using the multiplatform game development software Unity3D (version 4.2.1; Unity Technologies, San Francisco, CA, U.S.A.) running on a Windows 7 operating system with an i7 processor and NVIDIA GTX 660 graphics card. On this system, Unity3D rendered at a rate of approximately 60 frames/s. We selected Unity3D as our software platform because it permits dynamic design of 3D stimuli and environments, allows full control of subject/object interaction and can be set up to communicate with external devices. Simulated environments were created using a virtual four-camera cube rig to reflect the position of the tested spider. The rig consisted of top (facing up) and bottom (facing down) cameras, as well as left and right cameras positioned at  $\pm 45^\circ$  degrees to the spider's forward-facing centre. The images output through the four cube faces resulted in a 90-degree perspective. To solve for a 180-degree fish-eye projection, the images output from the four cube faces were applied as textures to four objects (known as 'meshes' in Unity), producing a single image predistorted to appear normal against the hemispherical projection geometry (Bourke, 2009).

Two closed-loop scenes were created for the experiments described below. Both environments contained a flat ground plane that was textured and coloured to resemble tree bark. In preliminary trials we found that spiders displayed limited interaction with the simulation when the ground was entirely uniform in appearance and lacking in texture. Lycosid spiders attend to the visual structure of the substratum while navigating home (Ortega-Escobar, 2011), and it is likely that substratum appearance is also relevant to salticid spiders, presumably by contributing to perceived ventral optic flow (Ortega-Escobar & Ruiz, 2014).

An interactive graphical user interface was created to calibrate, run and play back our simulations. To calibrate distance in VR, we measured a single rotation of the treadmill ball and used this value to scale the virtual environment, so that 1 cm travelled on the treadmill in the RW equalled 1 cm travelled in VR. The system was recalibrated at the start of each test day. We recorded each spider's mean speed and total distance travelled for all trials. In addition, we observed spatial behaviour and responses to objects in a real-time replay function, which played back the trial from the spider's point of view, as well as a 'static replay' function, which displayed a line trace of the path taken throughout the trial. We also created a data collection tool that could be 'attached' to virtual objects to assist in our estimation of the level of interaction with these objects. The data collection tool allowed us to set a radius around a given object and subsequently record the latency to enter this radius (approach

latency), number of entries and total amount of time spent in that region. Additionally, a 'dot product' function was included which, for each time point sampled, output a value ranging from  $-1$  to  $1$ , where  $-1$  denotes that the spider is directly facing the object,  $1$  denotes that the spider is facing directly away from the object, and  $0$  denotes that the spider is facing  $90^\circ$  away from the object. Multiple areas of interest could be set on a single object, and multiple objects could be set as objects of interest, collectively providing high-resolution output on the spider's orientation throughout the trial.

#### Experiment 1: Behaviour in RW and VR

To test whether interindividual behaviour differences were conserved across contexts we measured spontaneous locomotor activity and light/dark preference of adult female spiders ( $N = 32$ ) in both RW and VR. Activity levels are often highly repeatable within species (Richardson, 2001) and have been found to correlate with other behaviours in spiders (Pruitt, Riechert, & Jones, 2008). We started RW locomotor activity testing 24 h after attachment of the magnet. Spiders were placed individually into glass petri dishes (15 cm diameter) and spontaneous locomotion was assessed for 10 min. Trials were videorecorded from above using a digital video camera (Panasonic HDC-SD900). The total distance travelled was measured from these recordings using LoliTrack 2.0 (Loligo Systems, Tjele, Denmark). To test whether affixing or presence of the magnet affected mobility, locomotion of a randomly selected subset of 11 spiders was also recorded and analysed 24 h before affixing the magnet. For these spiders, comparisons were made between pre- and postmagnet activity measures.

The light/dark preference test is commonly used to assess associative behaviours in invertebrates (Steenbergen, Richardson, & Champagne, 2011), including arachnids (Camp & Gaffin, 1999; Dos Santos, Hogan, & Willemart, 2013). RW dark preference testing began approximately 24 h after locomotor activity testing with the same group of spiders. Spiders were placed in a rectangular arena ( $7.5 \times 15$  cm and 7.5 cm high) constructed from white plastic board (Corflute). The walls of the arena were lightly dusted with nonscented talcum powder to prevent spiders from escaping, and the top was open to permit video recording. A black piece of card was placed over one half of the arena creating a shaded 'dark' zone underneath. Trials lasted 20 min and were initiated by placing the spider in the centre of the 'light' zone. All trials were video-recorded from above and subsequently scored from these recordings. We assessed the latency until spiders first entered the dark side of the arena, as well as the proportion of each trial spent on the dark side.

VR locomotor activity and dark preference were tested in a single, combined session approximately 24 h after RW dark testing (see above for general procedures). The VR environment was a square arena (52 cm wide, 22 cm high), designed to be either 'empty', containing no objects, or 'complex', containing 18 randomly placed pillars (3 cm diameter, 8 cm high). The virtual arena was scaled to a larger size than the corresponding RW arena in order to reduce the probability of spiders reaching a virtual wall, which would not possess the properties of a physical wall and could create breaks in simulation 'reality' (Sherman & Craig, 2002). As in the RW, spiders began a trial in the centre of the 'light' zone. We measured the relative ambient intensity contrast ( $W/m^2$  per nm) of the light region compared to the dark region using a JAZ EL-200 portable spectrometer fitted with a spectralon diffused irradiance module (Ocean Optics Ltd., Dunedin, FL, U.S.A.). The light region was approximately five to six times brighter than the dark region in both environments (real world

1:5.9; VR 1:4.9). Initially, spiders were assigned evenly to either VR condition, but we shifted this allocation in favour of the complex treatment (empty,  $N = 11$ ; complex,  $N = 21$ ) after observing abnormal behaviour in spiders in the empty condition (see Results).

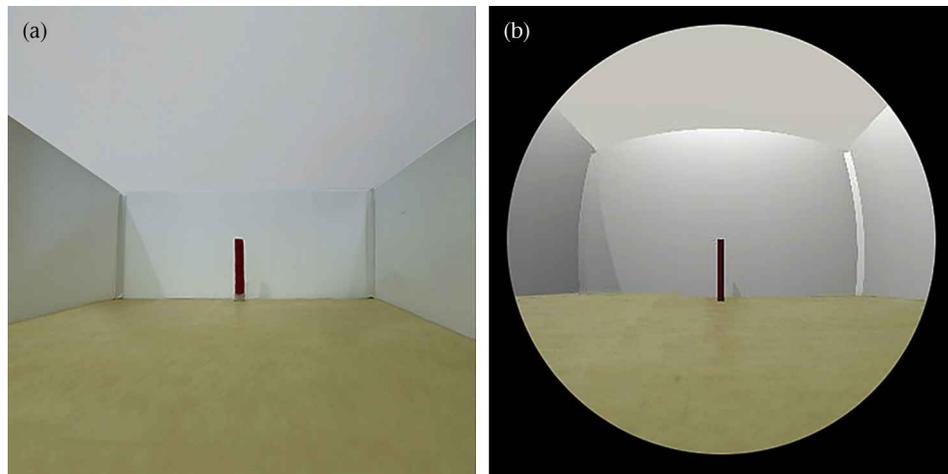
#### Experiment 2: Beacon Learning

Salticids construct silken nests for shelter (Jackson, 1979) and commonly exhibit high nest site fidelity (Hoeffler & Jakob, 2006; Jackson, 1988). An experiment was conducted with a new group of spiders to determine (1) whether spiders attended to beacons previously paired with a nest site, and (2) whether and how RW experiences influence behaviour in VR.

Following attachment of the magnet, spiders were placed in individual petri dishes (1.5 cm high, 15 cm diameter) and given 48 h to build a nest under a folded sheet of paper ( $2 \times 3$  cm) that was shaped as a 'tent'. On the second day in the petri dish, and every second day following, spiders received one Queensland fruit fly as food. Spiders were then transferred in their nests to square individual 'home arenas' (52 cm wide, 22 cm high) that they occupied for the duration of the experiment. Nest sites were placed 7.5 cm from the middle of one wall. The arena walls were constructed from white plastic board (Corflute) that was dusted with nonscented talcum powder to prevent spiders from escaping. Between trials, the arena was covered with a translucent polypropylene sheet fitted to the top of the walls that prevented spiders from seeing out of their enclosures while allowing diffuse illumination. Spiders were divided into two groups based on whether a beacon was present. 'Beacon spiders' ( $N = 12$ ) had a beacon (a red pillar or green cross) placed immediately behind their nest site, while 'No Beacon spiders' ( $N = 12$ ) had no beacon present. All spiders underwent an identical test protocol of five RW trials and two VR trials: two RW trials on each of days 1 and 2, separated by 3 h, one VR trial on each of days 3 and 4 and a single RW trial immediately following the final VR trial on day 4. The fifth RW trial was included to test whether 2 days of testing in VR had an effect on subsequent behaviour in the RW.

For RW trials, spiders were transferred into clean arenas that were identical to the arenas they had occupied previously with a beacon present but no nest (Fig. 2a). For Beacon spiders, the beacon was the same type that was present in their home arenas. No Beacon spiders, which had no prior exposure to a beacon, were assigned one of the two beacon types and were subsequently tested with this beacon in all trials. The beacon was placed 7.5 cm from the middle of one wall (as in home arenas), with a different wall randomly selected on each trial to prevent spiders from orienting to global cues beyond the arena. Spiders were given 3 min to acclimate while restrained in the centre of the arena by an upturned opaque vial before the vial was raised and the trial began. Each trial lasted 20 min and was video-recorded from above.

The VR environment was a square arena designed to be similar in appearance and scale to the RW arena. The VR arena contained a single beacon in the same position relative to the spider's starting position as in the RW (Fig. 2b). We measured the relative radiance contrast ( $W/m^2$  per nm per sr) of the beacon against the white wall in both environments. The background was approximately twice as 'bright' as the beacon in both worlds (beacon:background: real world 1:2; VR 1:1.5). Spiders began all trials in the centre of the arena facing the beacon, and all trials lasted 20 min. The treadmill was wiped with a damp cloth between trials to remove chemical cues left behind by the preceding spider (Jackson, 1987).



**Figure 2.** Spider's starting perspective in the beacon experiment in (a) real and (b) virtual environments. The virtual view reflects the image pretransformed to fill the hemispherical display screen.

Spiders were scored for two measures in both contexts, recorded when the spider entered a 7.5 cm radius circle surrounding the beacon: the proportion of the trial spent in proximity to the beacon and the number of beacon visits. In addition, total path length was recorded for spiders in the VR environment as a measure of general activity levels. We adopted an absolute proximity radius for scoring in order to have an objective measure of approach behaviours across trials. A radius of 7.5 cm was selected on the basis of data from pilot trials.

### Analyses

Statistical analyses were performed using JMP version 11 (SAS Institute Inc., Cary, NC, U.S.A.). We performed nonparametric statistics for all analyses as our data did not meet the assumptions of normality. For experiment 1, descriptive data are presented for the empty VR arenas but due to abnormal behaviour this group was excluded from subsequent analyses. Spiders that did not participate in this task, defined here as path length of less than 30 cm in the 20 min trial, were also excluded ( $N = 2$ ). A Wilcoxon matched-pairs test was used to test for differences between RW activity and VR activity for spiders in complex VR arenas. Spearman rank correlations were used to assess association between RW and VR in distance travelled, approach latency to enter the dark compartment and proportion of the trial spent in the dark compartment. A Wilcoxon matched-pairs test was used to test for differences in RW activity before and after attaching the magnet.

For experiment 2, RW and VR conditions were analysed separately and then compared to identify consistency in performance across contexts. Wilcoxon matched-pairs tests were used to compare the mean time spent in proximity to the beacon and the number of beacon visits in the RW and VR, and Wilcoxon signed-ranks tests were used to detect group differences in performance on both measures within both RW and VR contexts. In the RW, a Friedman's test was used to test for a trial effect in both measures across trials 1–4. A separate test was run comparing trials 4 and 5 to investigate whether VR testing had any effect on subsequent RW behaviour. Spearman rank correlations were used to assess association between RW and VR for each performance measure (outlined above). For the VR trials we additionally compared the total distance travelled by Beacon and No Beacon spiders using a Wilcoxon signed-ranks test.

### Ethical Note

We followed the ABS/ASAB guidelines for the ethical treatment of animals. Attachment of the magnet in advance of experiments minimized stressful handling and allowed us to return spiders to their home cages after each trial. Feeding, behaviour and survival were not visibly affected by attachment of the magnet or participation in experiments. Spiders were subsequently used in pilot trials for other VR experiments.

## RESULTS

### Experiment 1: Behaviour in RW and VR

In VR, spiders in empty virtual arenas, containing no objects, were significantly less active than spiders in complex arenas (mean distance travelled  $\pm$  SE: complex =  $160.3 \pm 18.4$  cm; empty =  $92.5 \pm 25.4$  cm;  $Z = -2.40$ ,  $P = 0.016$ ), often stopping for prolonged periods during the trial. Owing to their substantial reduction in general activity levels, spiders from the empty condition were excluded from further analyses.

We found a significant positive correlation between each RW measure and its corresponding VR measure, although spiders tended to travel shorter distances in VR, were quicker to enter the dark compartment, and spent a greater proportion of the trial on the dark side of the arena than when in the RW (Table 1). Attachment of the magnet did not significantly influence activity levels in our RW activity trial (mean distance travelled in 10 min trials:

**Table 1**  
Statistical comparison of measures in the RW and VR in experiment 1

Measure	Means $\pm$ SE		Wilcoxon matched-pairs		Spearman rank correlation	
	RW	VR	Z	P	$r_s$	P
Distance	383.8 $\pm$ 38.8	160.3 $\pm$ 18.6	-4.02	0.001	0.579	0.006
Approach latency	466 $\pm$ 99.1	188.6 $\pm$ 76.1	-2.94	0.003	0.637	0.002
Proportion	0.50 $\pm$ 0.08	0.67 $\pm$ 0.09	-2.21	0.027	0.473	0.031

Differences between each measure in the RW and its corresponding measure in VR were analysed with Wilcoxon matched-pairs tests. Correlations between each measure in the RW and its corresponding measure in VR were analysed with Spearman rank correlations. Distance refers to the total distance travelled; approach latency refers to the time to first enter the dark side of the arena and proportion refers to the proportion of the trial spent in the dark side (mean  $\pm$  SE,  $N = 21$ ).

premagnet =  $407.9 \pm 46$  cm; postmagnet =  $390.5 \pm 48.1$  cm;  $Z = -0.46$ ,  $P = 0.644$ ).

### Experiment 2: Beacon Learning

In the RW, Beacon spiders, which had previously been exposed to a beacon beside their nest site in their home arena, made more visits to a beacon and spent significantly more time in its proximity than No Beacon spiders when tested in clean arenas (visits:  $Z = 2.24$ ,  $P = 0.025$ ; time in proximity:  $Z = 3.30$ ,  $P = 0.001$ ). There was no evidence of a difference across the four initial RW trials (visits:  $\chi^2_3 = 6.79$ ,  $P = 0.080$ ; time in proximity:  $\chi^2_3 = 1.80$ ,  $P = 0.615$ ) or between RW trial 4 (the trial preceding VR testing) and 5 (the RW trial immediately following VR testing; visits:  $\chi^2_1 = 2.57$ ,  $P = 0.109$ ; time in proximity:  $\chi^2_1 = 1.19$ ,  $P = 0.275$ ). The similarity in performance between RW trials 4 and 5 indicates that the intervening VR trials did not disrupt subsequent performance in the RW.

The time spent in proximity to the beacon was positively correlated between the RW and VR for Beacon spiders but not for No Beacon spiders, suggesting that learning of the association between beacons and nest sites transferred across contexts (Table 2).

As was the case in the RW, when tested in VR Beacon spiders spent significantly more time in proximity to the virtual beacon than did the No beacon spiders ( $Z = 2.81$ ,  $P = 0.005$ ), although there was no significant difference in the number of visits ( $Z = 1.50$ ,  $P = 0.128$ ; Fig. 3).

Beacon spiders had significantly longer path lengths in VR than No Beacon spiders (Beacon spiders =  $118 \pm 17$  cm; No Beacon spiders =  $57.4 \pm 16$  cm;  $Z = 2.20$ ,  $P = 0.028$ ). For both Beacon and No Beacon spiders, activity peaked during the first 120 s and gradually declined over the course of the trial (Fig. 4).

## DISCUSSION

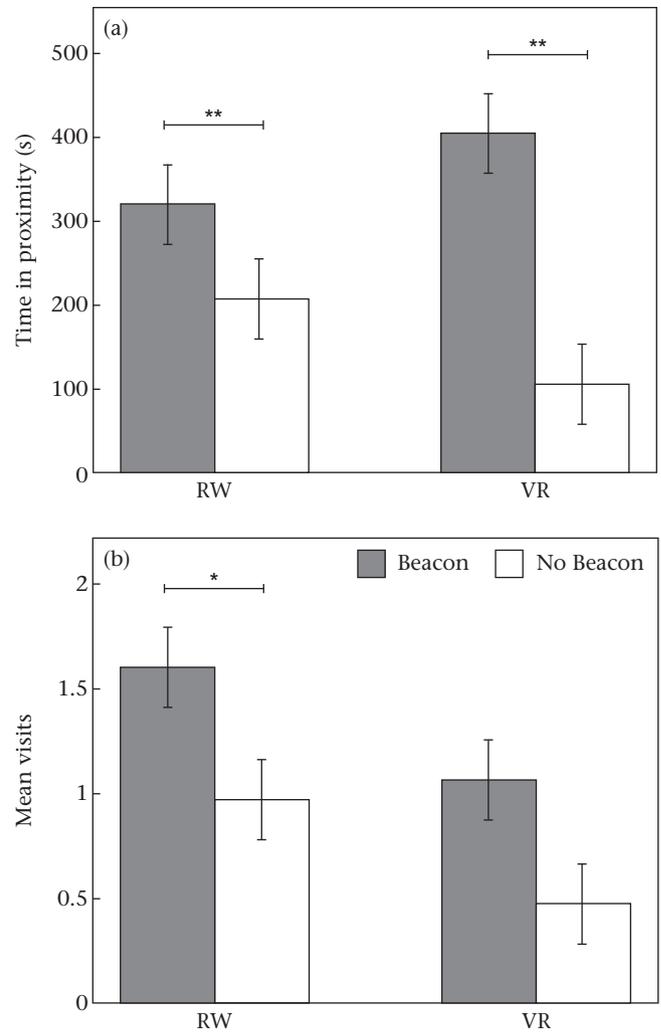
Video and computer-animated stimuli have been presented to salticids in order to study psychophysics (Zurek, Taylor, Evans, & Nelson, 2010), cognition (Dolev & Nelson, 2014), courtship (Clark & Uetz, 1992), contests (Tedore & Johnsen, 2015) and predation (Bednarski et al., 2012), but these studies have always been conducted under open-loop conditions. The VR system described in the present study closes the loop for the first time, demonstrating that salticids detect and respond appropriately to the visual and motor feedback of a virtual environment. The present study comprises the first demonstration that spiders, here, female *S. incana* jumping spiders, display patterns of activity and behaviour in VR that closely parallel their RW counterparts. Individual tendencies were conserved across contexts: both individual locomotor activity levels and dark-seeking behaviour in the RW were positively correlated with locomotor activity and dark-seeking behaviour in VR. Further, associations learned in the RW transferred to VR.

**Table 2**

Statistical comparison of measures in the RW and VR in experiment 2

Measure	Group	RW	VR	Spearman rank correlation		Wilcoxon matched-pairs	
				$r_s$	$P$	$Z$	$P$
Time in proximity	Beacon	$320.2 \pm 43.2$	$404.4 \pm 104.6$	0.625	0.009	-0.78	0.437
	No Beacon	$206.9 \pm 49.7$	$105.1 \pm 43.2$	0.089	0.717		
No. of visits	Beacon	$1.6 \pm 0.2$	$1.1 \pm 0.3$	0.409	0.116	-3.09	0.002
	No Beacon	$1.0 \pm 0.1$	$0.5 \pm 0.1$	0.193	0.429		

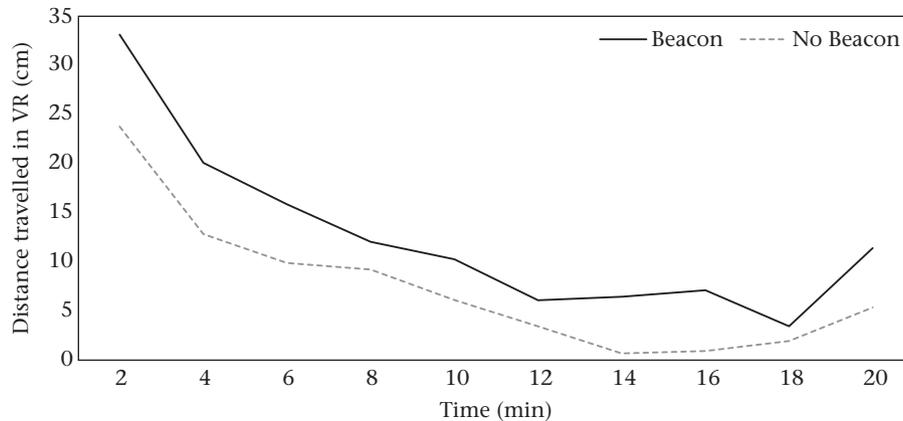
Correlations between each measure in the RW and its corresponding measure in VR were analysed with Spearman rank correlations. Differences between each measure in the RW and its corresponding measure in VR were analysed with Wilcoxon matched-pairs tests. Time in proximity refers to the amount of time (s) spent in proximity to the beacon and no. of visits refers to the number of approaches to the beacon ( $N = 35$ ).



**Figure 3.** Spider performance in a beacon learning task in the RW and VR. (a) Mean time spent in proximity to the beacon  $\pm$  SE. (b) Mean number of visits to the beacon  $\pm$  SE. Beacon spiders were trained in the RW with a beacon/nest site pairing while No Beacon spiders were not provided with a beacon. Asterisks denote significant differences between Beacon and No Beacon spiders. \* $P < 0.05$ ; \*\* $P < 0.001$ ; Wilcoxon signed-ranks test.

Spiders that were given the opportunity to learn about beacons associated with their nest site in the RW later tended towards these cues in both the RW and in VR.

Paying attention to local cues such as beacons is thought to be less computationally demanding than using distal cues (Shettleworth, 2010), and is part of the navigational strategy of many arthropods (Cheng, 2000; Collett, Graham, & Durier, 2003; Graham, 2003). A field study of a North American salticid,



**Figure 4.** Mean distance travelled by Beacon and No Beacon spiders over the course of a 20 min trial in the VR system. Time is grouped into 120 s time bins and each time point reflects the distance moved since the previous time point.

*Phiddipus clarus*, found that spiders readily learned a beacon–nest site association and showed an increased tendency to approach beacons that were displaced from their original location (Hoefler & Jakob, 2006). Despite geographical and habitat differences we found a similar tendency to learn cues associated with nest sites. These results suggest that local cues may be an ecologically and taxonomically widespread element of the navigational strategy used by salticids, especially for homing.

VR may be a particularly useful tool in the study of navigation. Beacons, landmarks and environmental cues can easily be removed, manipulated or put into conflict with each other, and these manipulations can occur alongside neurophysiological recordings (Bohil et al., 2011). VR has been used extensively to study the neural underpinnings of behaviour in restrained primates, and more recently in rodents. Both rodents and primates display hippocampal place cell activity when navigating in VR (primates: Hori et al. 2005; rodents: Hölscher, Schnee, Dahmen, Setia, & Mallot, 2005), but have critical differences in their activation patterns between real and virtual environments that can be attributed to lack of appropriate vestibular input in VR (Ravassard et al., 2013). Restraint can similarly lead to behavioural artefacts in invertebrate models that possess equilibrium sensors. Flies, for example, exhibit distortions in flight behaviour when mechanosensory feedback from specialized stabilizing organs, the gyroscopic halteres, is impeded, as is the case during restraint (Fry, Sayaman, & Dickinson, 2003; Pringle, 1948). The limitations imposed by equilibrium sensors can be circumvented through VR studies of visually orienting walking invertebrates that lack equilibrium-sensing organs, such as spiders.

Until recently, recording from salticid brains has been unfeasible owing to their highly pressurized internal fluids, and the resulting fluid loss that accompanies incision. A recent study has developed methods that overcome this limitation, obtaining the first neurophysiological recordings from the brain of a salticid as it views traditional grating stimuli and naturalistic prey-like stimuli that were presented using open-loop methods (Menda et al., 2014). The effectiveness of a VR system for salticids in the present study raises the possibility of recording from brain cells in alert salticids interacting with an immersive, closed-loop environment.

The VR system described in the present study was designed to focus solely on vision, which is well known to mediate diverse aspects of salticid behaviour (Jackson & Harland, 2009; Land, 1969a). At the same time, many species routinely use other sensory modalities, such as chemoreception and vibration, both to assess the environment and to communicate (Elias et al., 2012;

Girard, Kasumovic, & Elias, 2011; Jackson, 1987). A VR system could be designed to emphasize any modality, and indeed systems based on olfaction (Fry et al. 2008; Sakuma, 2002) and audition (Fry et al. 2004) have been used with other invertebrates. However, setting up a similar system for salticids would be challenging as salticids access these cues in part through the substrate (Barth, 2002; but see Jackson, Clark, & Harland, 2002; Jackson & Cross, 2011 for examples of airborne chemoception in salticids), and the ‘substrate’ in our set-up was an air-supported sphere atop which spiders were mounted. Although delivery of vibratory stimuli through this cushion of air would be particularly challenging, it would be straightforward to incorporate substrate-bound chemical cues applied to the ball prior to the start of each trial or to deploy puffs of airborne odours or airborne sound.

Irrespective of the modality emphasized in VR, it is important to ensure that the attributes present in the simulation are tuned to match the perceptual system of the subject. In a visual system, parameters such as size, shape, colour, texture, luminance and movement all require careful consideration (Woo & Rieucau, 2011; Zeil, 2000). The extensive body of research on visual perception of salticids provides an excellent framework for designing stimulus features that most effectively elicit a natural response. For instance, Zurek et al. (2010) demonstrated that the orientation response in *S. incana* is dependent on stimulus size, contrast and speed; Bednarski et al. (2012) highlighted the importance of motion characteristics in eliciting a predatory response; and Dolev and Nelson (2014) showed that the relative angle between the body elements of abstract stimuli are sufficient and necessary for recognition. VR will provide an excellent resource to build from this foundation, as it broadens the scope of experimental possibility to encompass design features and contingencies that were previously inaccessible in salticids.

#### Acknowledgments

We thank Rowan McGinley for his assistance collecting spiders, Bruno Van Swinderen for his help conceptualizing our VR system, Thomas White for providing spectral measurements and three anonymous referees for their helpful suggestions on the manuscript. We are grateful to Chris Malloy, Rob Lee, Brian Su, Keith Hassan and John Porte for their invaluable technical assistance. We are especially thankful to Greg Hunsburger for his assistance in designing and implementing the 3D environment, and his continual support throughout this project. This research was supported by an Australian Government Endeavour Postgraduate

Award. Research expenses were supported by Higher Degrees Research Funds of the Department of Biological Sciences at Macquarie University.

## References

- Barth, F. G. (2002). Spider senses—technical perfection and biology. *Zoology (Jena, Germany)*, 105(4), 271–285. <http://dx.doi.org/10.1078/0944-2006-00082>.
- Bednarski, J. V., Taylor, P. W., & Jakob, E. M. (2012). Optical cues used in predation by jumping spiders, *Phidippus audax* (Araneae, Salticidae). *Animal Behaviour*, 84(5), 1221–1227. <http://dx.doi.org/10.1016/j.anbehav.2012.08.032>.
- Bender, J. A., & Dickinson, M. H. (2006). A comparison of visual and haltere-mediated feedback in the control of body saccades in *Drosophila melanogaster*. *The Journal of Experimental Biology*, 209(Pt 23), 4597–4606. <http://dx.doi.org/10.1242/jeb.02583>.
- Bohil, C. J., Alica, B., & Biocca, F. (2011). Virtual reality in neuroscience research and therapy. *Nature Reviews. Neuroscience*, 12(12), 752–762. <http://dx.doi.org/10.1038/nrn3122>.
- Bourke, P. (2009). *iDome: Immersive gaming with the Unity3D game engine* (Vol. 1, pp. 265–272). Singapore: Computer Games, Multimedia and Allied Technology 09 (CGAT09). <http://dx.doi.org/10.1037/e602482011-012>.
- Brembs, B. (2000). The operant and the classical in conditioned orientation of *Drosophila melanogaster* at the flight simulator. *Learning & Memory*, 7(2), 104–115. <http://dx.doi.org/10.1101/lm.7.2.104>.
- Brembs, B. (2011). Spontaneous decisions and operant conditioning in fruit flies. *Behavioural Processes*, 87(1), 157–164. <http://dx.doi.org/10.1016/j.beproc.2011.02.005>.
- Camp, E. A., & Gaffin, D. D. (1999). Escape behavior mediated by negative phototaxis in the scorpion *Paruroctonus utahensis*. *American Arachnological Society*, 27(3), 679–684.
- Cheng, K. (2000). How honeybees find a place: lessons from a simple mind. *Animal Learning & Behavior*, 28(1), 1–15. <http://dx.doi.org/10.3758/BF03199768>.
- Clark, D. L., & Uetz, G. W. (1990). Video image recognition by the jumping spider, *Maevia inclemens* (Araneae: Salticidae). *Animal Behaviour*, 40(5), 884–890.
- Clark, D. L., & Uetz, G. W. (1992). Morph-independent mate selection in a dimorphic jumping spider: demonstration of movement bias in female choice using video-controlled courtship behaviour. *Animal Behaviour*, 43(2), 247–254. [http://dx.doi.org/10.1016/S0003-3472\(05\)80220-9](http://dx.doi.org/10.1016/S0003-3472(05)80220-9).
- Collett, T. S., Graham, P., & Durier, V. (2003). Route learning by insects. *Current Opinion in Neurobiology*, 13(6), 718–725. <http://dx.doi.org/10.1016/j.conb.2003.10.004>.
- Dahmen, H. J. (1980). A simple apparatus to investigate the orientation of walking insects. *Experientia*, 36(6), 685–687. <http://dx.doi.org/10.1007/BF01970140>.
- D'Eath, R. B. (1998). Can video images imitate real stimuli in animal behaviour experiments? *Biological Reviews*, 73, 267–292. <http://dx.doi.org/10.1111/j.1469-185X.1998.tb00031>.
- Dill, M., Wolf, R., & Heisenberg, M. (1993). Visual pattern recognition in *Drosophila* involves retinotopic matching. *Nature*, 365(6448), 751–753.
- Dolev, Y., & Nelson, X. J. (2014). Innate pattern recognition and categorization in a jumping spider. *PLoS One*, 9(6), e97819. <http://dx.doi.org/10.1371/journal.pone.0097819>.
- Dombeck, D. A., & Reiser, M. B. (2012). Real neuroscience in virtual worlds. *Current Opinion in Neurobiology*, 22(1), 3–10. <http://dx.doi.org/10.1016/j.conb.2011.10.015>.
- Dos Santos, G. C., Hogan, J. A., & Willemart, R. H. (2013). Associative learning in a harvestman (Arachnida, Opiliones). *Behavioural Processes*, 100, 64–66. <http://dx.doi.org/10.1016/j.beproc.2013.07.021>.
- Elias, D. O., Land, B. R., Mason, A. C., & Hoy, R. R. (2006). Measuring and quantifying dynamic visual signals in jumping spiders. *Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural, and Behavioral Physiology*, 192(8), 785–797. <http://dx.doi.org/10.1007/s00359-006-0116-7>.
- Elias, D. O., Maddison, W. P., Peckmezian, C., Girard, M. B., & Mason, A. C. (2012). Orchestrating the score: complex multimodal courtship in the *Habronattus coecatus* group of *Habronattus* jumping spiders (Araneae: Salticidae). *Biological Journal of the Linnean Society*, 105(3), 522–547. <http://dx.doi.org/10.1111/j.1095-8312.2011.01817.x>.
- Fleishman, L. J., & Endler, J. A. (2000). Some comments on visual perception and the use of video playback in animal behavior studies. *Acta Ethologica*, 3(1), 15–27. <http://dx.doi.org/10.1007/s102110000025>.
- Fleishman, L. J., McClintock, W., D'Eath, R., Brainard, D., & Endler, J. (1998). Colour perception and the use of video playback experiments in animal behaviour. *Animal Behaviour*, 56(4), 1035–1040. <http://dx.doi.org/10.1006/anbe.1998.0894>.
- Foelix, R. F. (2011). *Biology of Spiders. Insect systematics & evolution* (3rd ed., Vol. 14, pp. 102–196). New York, NY: Oxford University Press.
- Forster, L. M. (1985). Target discrimination in jumping spiders (Araneae: Salticidae). In F. G. Barth (Ed.), *Neurobiology of arachnids* (pp. 249–274). Berlin, Germany: Springer-Verlag.
- Fry, S. N., Müller, P., Baumann, H. J., Straw, A. D., Bichsel, M., & Robert, D. (2004). Context-dependent stimulus presentation to freely moving animals in 3D. *Journal of Neuroscience Methods*, 135(1–2), 149–157. <http://dx.doi.org/10.1016/j.jneumeth.2003.12.012>.
- Fry, S. N., Rohrseitz, N., Straw, A. D., & Dickinson, M. H. (2008). TrackFly: virtual reality for a behavioral system analysis in free-flying fruit flies. *Journal of Neuroscience Methods*, 171(1), 110–117. <http://dx.doi.org/10.1016/j.jneumeth.2008.02.016>.
- Fry, S. N., Sayaman, R., & Dickinson, M. H. (2003). The aerodynamics of free-flight maneuvers in *Drosophila*. *Science*, 300(5618), 495–498.
- Girard, M. B., Kasumovic, M. M., & Elias, D. O. (2011). Multi-modal courtship in the peacock spider, *Maratus volans* (O.P.-Cambridge, 1874). *PLoS One*, 6(9), e25390. <http://dx.doi.org/10.1371/journal.pone.0025390>.
- Gotz, B. Y. K. G. (1987). Course-control, metabolism and wing interference during ultralong tethered flight in *Drosophila melanogaster*. *Journal of Experimental Biology*, 46(128), 35–46.
- Graham, P. (2003). The influence of beacon-aiming on the routes of wood ants. *Journal of Experimental Biology*, 206(3), 535–541. <http://dx.doi.org/10.1242/jeb.00115>.
- Gray, J. R., Pawlowski, V., & Willis, M. A. (2002). A method for recording behavior and multineuronal CNS activity from tethered insects flying in virtual space. *Journal of Neuroscience Methods*, 120(2), 211–223. <http://www.ncbi.nlm.nih.gov/pubmed/12385771>.
- Harland, D. P., & Jackson, R. R. (2002). Influence of cues from the anterior medial eyes of virtual prey on *Portia fimbriata*, an araneophagic jumping spider. *Journal of Experimental Biology*, 205(13), 1861–1868.
- Harland, D. P., & Jackson, R. R. (2004). *Portia* perceptions: the Umwelt of an araneophagic jumping spider. In F. R. Prete (Ed.), *Complex worlds from simpler nervous systems* (pp. 5–40). Cambridge, MA: MIT Press.
- Hoefler, C. D., & Jakob, E. M. (2006). Jumping spiders in space: movement patterns, nest site fidelity and the use of beacons. *Animal Behaviour*, 71(1), 109–116. <http://dx.doi.org/10.1016/j.anbehav.2005.03.033>.
- Hölscher, C., Schnee, A., Dahmen, H. J., Setia, L., & Mallot, H. A. (2005). Rats are able to navigate in virtual environments. *The Journal of Experimental Biology*, 208(3), 561–569. <http://dx.doi.org/10.1242/jeb.01371>.
- Hori, E., Nishio, Y., Kazui, K., Umeno, K., Tabuchi, E., Sasaki, K., et al. (2005). Place-related neural responses in the monkey hippocampal formation in a virtual space. *Hippocampus*, 15(8), 991–996. <http://dx.doi.org/10.1002/hipo.20108>.
- Jackson, R. R. (1979). Nests of *Phidippus johnsoni* (Araneae, Salticidae): characteristics, pattern of occupation and function. *Journal of Arachnology*, 7(1), 47–58. <http://dx.doi.org/10.2307/3704953>.
- Jackson, R. R. (1987). Comparative study of releaser pheromones associated with the silk of jumping spiders (Araneae, Salticidae). *New Zealand Journal of Zoology*, 14(1), 1–10. <http://dx.doi.org/10.1080/03014223.1987.10422676>.
- Jackson, R. R. (1988). The biology of *Jacksonoides queenslandica*, a jumping spider (Araneae: Salticidae) from Queensland: intraspecific interactions, web-invasion, predators, and prey. *New Zealand Journal of Zoology*, 15(1), 1–37. <http://dx.doi.org/10.1080/03014223.1988.10422606>.
- Jackson, R. R., Clark, R. J., & Harland, D. P. (2002). Behavioural and cognitive influences of kairomones on an araneophagic jumping spider. *Behaviour*, 139(6), 749–775. <http://dx.doi.org/10.1163/156853902320262808>.
- Jackson, R. R., & Cross, F. R. (2011). Olfaction-based mate-odor identification by jumping spiders from the genus *Portia*. *Journal of Zoology*, 39(3), 439–443.
- Jackson, R. R., & Cross, F. R. (2013). A cognitive perspective on aggressive mimicry. *Journal of Zoology*, 290(3), 161–171. <http://dx.doi.org/10.1111/jzo.12036>.
- Jackson, R. R., & Harland, D. P. (2009). One small leap for the jumping spider but a giant step for vision science. *Journal of Experimental Biology*, 212, 2129–2132.
- Jackson, R. R., & Nelson, X. J. (2011). Reliance on trial and error signal derivation by *Portia africana*, an araneophagic jumping spider from East Africa. *Journal of Ethology*, 29(2), 301–307. <http://dx.doi.org/10.1007/s10164-010-0258-5>.
- Jackson, R. R., & Pollard, S. D. (1996). Predatory behavior of jumping spiders. *Annual Review of Entomology*, 41, 287–308. <http://dx.doi.org/10.1146/annurev.en.41.010196.001443>.
- Jakob, E. M., Skow, C. D., Haberman, M. P., & Plourde, A. (2007). Jumping spiders associate food with color cues in a t-maze. *Journal of Arachnology*, 35(3), 487–492. <http://dx.doi.org/10.1636/JOA-ST06-61.1>.
- Labhart, T., & Nilsson, D. E. (1995). The dorsal eye of the dragonfly *Sympetrum*: specializations for prey detection against the blue sky. *Journal of Comparative Physiology A*, 176(4), 437–453. <http://dx.doi.org/10.1007/BF00196410>.
- Land, M. F. (1969a). Movements of the retinae of jumping spiders (Salticidae: Dendryphantinae) in response to visual stimuli. *Journal of Experimental Biology*, 51, 471–493.
- Land, M. F. (1969b). Structure of the retinae of the principle eyes of jumping spiders (Salticidae: Dendryphantinae) in relation to visual optics. *Journal of Experimental Biology*, 51, 443–470.
- Land, M. F. (1971). Orientation by jumping spiders in the absence of visual feedback. *Journal of Experimental Biology*, 54(1), 119–139.
- Land, M. F. (1972). Stepping movements made by jumping spiders during turns mediated by the lateral eyes. *Journal of Experimental Biology*, 57(1), 15–40.
- Land, M. F. (2005). The optical structures of animal eyes. *Current Biology*, 15(9), 319–323. <http://dx.doi.org/10.1016/j.cub.2005.04.041>.
- Land, M. F., & Nilsson, D. E. (2012). *Animal eyes, second edition* (2nd ed.). Oxford, U.K.: Oxford University Press.
- Laughlin, S. B., de Ruyter van Steveninck, R. R., & Anderson, J. C. (1998). The metabolic cost of neural information. *Nature Neuroscience*, 1(1), 36–41. <http://dx.doi.org/10.1038/236>.
- McGinley, R. H., Prenter, J., & Taylor, P. W. (2015). Assessment strategies and decision making in male-male contests of *Servaeia incana* jumping spiders. *Animal Behaviour*, 101, 89–95. <http://dx.doi.org/10.1016/j.anbehav.2014.12.014>.

- Menda, G., Shamble, P. S., Nitzany, E. I., Golden, J. R., & Hoy, R. R. (2014). Visual perception in the brain of a jumping spider. *Current Biology*, 24(21), 1–6. <http://dx.doi.org/10.1016/j.cub.2014.09.029>.
- Nagata, T., Koyanagi, M., Tsukamoto, H., Saeki, S., Isono, K., Shichida, Y., et al. (2012). Depth perception from image defocus in a jumping spider. *Science*, 335(6067), 469–471. <http://dx.doi.org/10.1126/science.1211667>.
- Nakamura, T., & Yamashita, S. (2000). Learning and discrimination of colored papers in jumping spiders (Araneae: Salticidae). *Journal of Comparative Physiology*, 186(9), 897–901.
- Ortega-Escobar, J. (2011). Anterior lateral eyes of *Lycosa tarantula* (Araneae: Lycosidae) are used during orientation to detect changes in the visual structure of the substratum. *Journal of Experimental Biology*, 214(Pt 14), 2375–2380. <http://dx.doi.org/10.1242/jeb.055988>.
- Ortega-Escobar, J., & Ruiz, M. A. (2014). Visual odometry in the wolf spider *Lycosa tarantula* (Araneae: Lycosidae). *The Journal of Experimental Biology*, 217(3), 395–401. <http://dx.doi.org/10.1242/jeb.091868>.
- Peaslee, A. G., & Wilson, G. (1989). Spectral sensitivity in jumping spiders. *Journal of Comparative Physiology A*, 9(164), 359–363.
- Pringle, J. W. S. (1948). The gyroscopic mechanism of the halteres of Diptera. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 233(602), 347–384. <http://dx.doi.org/10.1098/rstb.1948.0007>.
- Pruitt, J. N., Riechert, S. E., & Jones, T. C. (2008). Behavioural syndromes and their fitness consequences in a socially polymorphic spider, *Anelosimus studiosus*. *Animal Behaviour*, 76(3), 871–879. <http://dx.doi.org/10.1016/j.anbehav.2008.05.009>.
- Ravassard, P., Kees, A., Willers, B., Ho, D., Aharoni, D. B., Cushman, J. D., et al. (2013). Multisensory control of hippocampal spatiotemporal selectivity. *Science (New York, N.Y.)*, 340(6138), 1342–1346. <http://dx.doi.org/10.1126/science.1232655>.
- Reiser, M. B., & Dickinson, M. H. (2010). *Drosophila* fly straight by fixating objects in the face of expanding optic flow. *The Journal of Experimental Biology*, 213(10), 1771–1781. <http://dx.doi.org/10.1242/jeb.035147>.
- Richardson, J. M. L. (2001). A comparative study of activity levels in larval Anurans and response to the presence of different predators. *Behavioral Ecology*, 12(1), 51–58. <http://dx.doi.org/10.1093/oxfordjournals.beheco.a000378>.
- Richardson, B. J., & Gunter, N. L. (2012). Revision of Australian jumping spider genus *Servaea* Simon 1887 (Araneae: Salticidae) including use of DNA sequence data and predicted distributions. *Zootaxa*, 33, 1–33.
- Sakuma, M. (2002). Virtual reality experiments on a digital servosphere: guiding male silkworm moths to a virtual odour source. *Computers and Electronics in Agriculture*, 35(2–3), 243–254. [http://dx.doi.org/10.1016/S0168-1699\(02\)00021-2](http://dx.doi.org/10.1016/S0168-1699(02)00021-2).
- Sherman, W. R., & Craig, A. B. (2002). *Understanding virtual reality: Interface, application, and design*. San Francisco, CA: Morgan Kaufmann.
- Shettleworth, S. J. (2010). *Cognition, evolution, and behaviour* (2nd ed.). New York, NY: Oxford University Press.
- Skow, C. D., & Jakob, E. M. (2006). Jumping spiders attend to context during learned avoidance of aposematic prey. *Behavioral Ecology*, 17(1), 34–40. <http://dx.doi.org/10.1093/beheco/ari094>.
- Steenbergen, P. J., Richardson, M. K., & Champagne, D. L. (2011). Patterns of avoidance behaviours in the light/dark preference test in young juvenile zebrafish: a pharmacological study. *Behavioural Brain Research*, 222(1), 15–25. <http://dx.doi.org/10.1016/j.bbr.2011.03.025>.
- Strauss, R., Schuster, S., & Götz, K. G. (1997). Processing of artificial visual feedback in the walking fruit fly *Drosophila melanogaster*. *The Journal of Experimental Biology*, 200(9), 1281–1296. <http://www.ncbi.nlm.nih.gov/pubmed/9172415>.
- Takalo, J., Piironen, A., Honkanen, A., Lempeä, M., Aikio, M., Tuukkanen, T., et al. (2012). A fast and flexible panoramic virtual reality system for behavioural and electrophysiological experiments. *Scientific Reports*, 2(1), 324. <http://dx.doi.org/10.1038/srep00324>.
- Tarsitano, M., & Jackson, R. R. (1997). Araneophagic jumping spiders discriminate between detour routes that do and do not lead to prey. *Animal Behaviour*, 53(2), 257–266. <http://dx.doi.org/10.1006/anbe.1996.0372>.
- Taylor, P. W., Hasson, O., & Clark, D. L. (2001). Initiation and resolution of jumping spider contests: roles for size, proximity, and early detection of rivals. *Behavioral Ecology and Sociobiology*, 50(5), 403–413. <http://dx.doi.org/10.1007/s002650100390>.
- Tedore, C., & Johnsen, S. (2015). Visual mutual assessment of size in male *Lysosomes viridis* jumping spider contests. *Behavioral Ecology*, 26(2), 510–518. <http://dx.doi.org/10.1093/beheco/aru222>.
- Wolf, R., Wittig, T., Liu, L., Wustmann, G., Eyding, D., & Heisenberg, M. (1998). *Drosophila* mushroom bodies are dispensable for visual, tactile, and motor learning. *Learning & Memory*, 5(1), 166–178. <http://dx.doi.org/10.1101/lm.5.1.166>.
- Woo, K. L., & Rieuecau, G. (2011). From dummies to animations: a review of computer-animated stimuli used in animal behavior studies. *Behavioral Ecology and Sociobiology*, 65(9), 1671–1685. <http://dx.doi.org/10.1007/s00265-011-1226-y>.
- Zeil, J. (2000). Depth cues, behavioural context, and natural illumination: some potential limitations of video playback techniques. *Acta Ethologica*, 3(1), 39–48. <http://dx.doi.org/10.1007/s102110000021>.
- Zurek, D. B., & Nelson, X. J. (2012a). Hyperacute motion detection by the lateral eyes of jumping spiders. *Vision Research*, 66, 26–30. <http://dx.doi.org/10.1016/j.visres.2012.06.011>.
- Zurek, D. B., & Nelson, X. J. (2012b). Saccadic tracking of targets mediated by the anterior-lateral eyes of jumping spiders. *Journal of Comparative Physiology. A, Sensory, Neural, and Behavioral Physiology*, 198(6), 411–417. <http://dx.doi.org/10.1007/s00359-012-0719-0>.
- Zurek, D. B., Taylor, A. J., Evans, C. S., & Nelson, X. J. (2010). The role of the anterior lateral eyes in the vision-based behaviour of jumping spiders. *Journal of Experimental Biology*, 213(14), 2372–2378. <http://dx.doi.org/10.1242/jeb.042382>.