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SPiDbox: A low-cost, design and validation of an open-source “Skinner-box” system for the study of jumping spiders

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SPiDbox: a low-cost, design and validation of an open-source “Skinner-box” system for the study of jumping spiders.

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Highlights

- Jumping spiders' learning abilities have proven difficult to test easily
- Here presented is an open-source, automated Skinner-box system
- It will allow for more standardized and objective experiments
- System validation demonstrates its effectiveness
- Future studies may employ it to test the jumping spiders' cognition

Abstract

1.1 Background

Skinner-box systems are fundamental in behavioural research. They are objective, reliable and can be used to carry out procedures otherwise impossible with manual methodologies. Recently, jumping spiders have caught the interest of scientists for their remarkable cognitive abilities. However, inquiries on their learning abilities are still few, since we lacked a proper methodology capable of overcoming the inherent difficulties that this family poses when carrying out a conditioning protocol.

1.2 New method

In this paper, a new, automated, open-source Skinner-box, intended for the study of jumping spiders is presented. The system is 3d printable, cheap, fully open-source; is controlled with a Raspberry Pi Zero by a Python script. Since spiders are too lightweight to activate large physical object, the SPiDbox employs photo-sensors.

1.3 Results

To validate the methodology, 30 *Phidippus regius* underwent a training procedure for a simple discrimination task to validate the effectiveness of the system. The spiders managed to learn the task, establishing the effectiveness of the SPiDbox.

1.4 Comparison with existing methods

This automated training appears to be more reliable and effective than traditional methodologies. Moreover, its highly scalable, as many SPiDboxes could be used in parallel.

1.5 Conclusions

The SPiDbox appears to be an effective system to train jumping spiders, opening up the possibility to study learning in increasingly more complex tasks, possibly extending our understanding of jumping spiders' cognitive abilities.

Keywords: operant conditioning, Skinner-box, jumping spider, learning, invertebrate

2 Introduction

Objectivity is as crucial as often elusive in behavioural research. Most of the time, experiments carried out in the lab rely on human intervention, introducing possible confounding factors arising from the experimenters' manipulation (Bohlen et al., 2014) or even by their mere presence (Sorge et al., 2014). Also, scoring procedures are generally carried out manually: this may not be a problem for simple binomial measures, but by increasing the complexity of the test, the scored behaviour may not be immediately evident (i.e. De Agrò et al., 2017), relying mostly on the potentially unconsciously biased experimenter. To solve this problem, double-blind procedures (Bello et al., 2014) can be implemented. However, these can be extremely costly in terms of time and resources.

A possible solution to this problem is the automation of both experimental procedures and data collection (Döring and Chittka, 2011). The invention of the first operant conditioning chamber (Skinner, 1938), the Skinner-box system, was an enormous scientific breakthrough in the study of

learning and conditioning. Today, Skinner-box-like systems are used throughout most animal research, especially for classical model species such as rodents, birds and monkeys. More recently many automated systems have been developed for the study of Hymenoptera, for tasks ranging from training (Brembs, 2003; Sokolowski and Abramson, 2010) to automated tracking (Crall et al., 2015; Stelzer and Chittka, 2010). These methodologies did permit higher objectivity and also greatly expanded the range of possibilities regarding what can be discovered through behavioural research (i.e. continuous recording of individual ant behaviour and interactions between colony members, impossible to achieve with manual techniques; Mersch et al., 2013). Machines can collect a massive amount of data, not comparable with what is achievable through manual procedures. Moreover, precision is improved, allowing the research to establish the exact reward contingencies and timing during training.

Among arthropods, more and more studies on jumping spiders cognitive abilities are being published (Herberstein, 2011), so much that the inquiries on the structure and functioning of their brain are ever-growing (Menda et al., 2014; Shamble et al., 2016; Steinhoff et al., 2019, 2018, 2017). They have been shown to possess numerical abilities (Cross and Jackson, 2017), behavioural flexibility (Cross and Jackson, 2015) and navigational abilities (Aguilar-Argüello et al., 2018, 2019; Cross and Jackson, 2016, 2019; Hill, 1979; Hoefler and Jakob, 2006; Peckmezian and Taylor, 2015a; Tarsitano, 2006; Tarsitano and Andrew, 1999; Tarsitano and Jackson, 1994, 1997). Moreover, their visual system has been extensively studied, from object recognition (Dolev and Nelson, 2016, 2014) to attention (Humphrey et al., 2018; Melrose et al., 2018).

However, even though conditioning experiments have been carried out (De Agrò et al., 2017; Liedtke and Schneider, 2014; Peckmezian and Taylor, 2015b; Skow and Jakob, 2006; Taylor et al., 2016), training jumping spiders remains a big challenge to overcome when studying this family (Jakob and Long, 2016). It has been suggested that one of the main reason why so many methodologies fail to find significant results is the animals' lack of motivation (Liedtke and Schneider, 2014; Jakob and Long, 2016). Spiders can survive for multiple weeks after having consumed a single prey (personal observation, but see also Guseinov et al., 2004: in nature, *Cyrtus algerina* had a prey in its chelicera in only 2.7% of all the sightings); moreover, they have a very brief period of activity during the day (Tarsitano and Jackson, 1997). The low energetic needs of jumping spiders prevent long-lasting procedures, forcing research to focus on simple S+/S- associations that require shorter, simpler training. Alternatively, longer training can be carried out, but with an immense expenditure of time and resources resulting in only a modest amount of data (De Agrò et al., 2017). To solve this problem, many authors used aversive conditioning methodologies (Bednarski et al., 2012; Long et al., 2015; Nakamura and Yamashita, 2000;

Peckmezian and Taylor, 2017, 2015b). These have the clear advantage of ensuring a constantly high motivation level, as the subjects will want to avoid the negative stimulus regardless of their other current motivation. However, training based on punishment has some inherent limitation: where in binomial choices reinforcing one or punishing the other have fundamentally the same effect, things changes when dealing with complex behavioural patterns or sequences of different behavioural responses. Animals can also sometimes adopt unforeseen behaviour in order to avoid punishment, rather than the one expected by the experimenter (Bednarski et al., 2012).

An automated training system would solve most of these problems, working continuously to catch the period of activity of the animals, being able to sustain prolonged training to cope with the low motivation of the animals, without having to rely on aversive stimuli. However, to my knowledge, no such procedure has ever been designed to test jumping spiders. To date, most experiments focusing on jumping spiders cognitive abilities have been carried out with spontaneous choice procedures, since our lack of reliable training methodologies prevented testing them outside of the natural domain.

In this paper, I present the SPiDbox: a Skinner-box system based on the Raspberry Pi. This system was intended to solve all of the above-mentioned problems, increasing reward contingencies precision and decreasing training times, while also requiring less human resources needed to carry them out.

3 Materials and methods

The SPiDbox was designed with 4 main requirements: it had to be easy to produce, easy to use, low cost and open source. All the components are cheap and readily available, or even 3d printable. Moreover, once built the machine can be operated by anybody, and the software can be easily modified to fit different reward routines. Source code, circuit design and 3d models are all available in a continuously updated repository on git-hub (<https://github.com/massimodeagro/SPiDbox>).

3.1 General structure

The SPiDbox (figure 1) is divided into three sections. The parts were all designed in openSCAD (version 2018.01.06) (Kintel and Wolf, 2011) and 3D printed in PLA plastic with a Creality CR-10.

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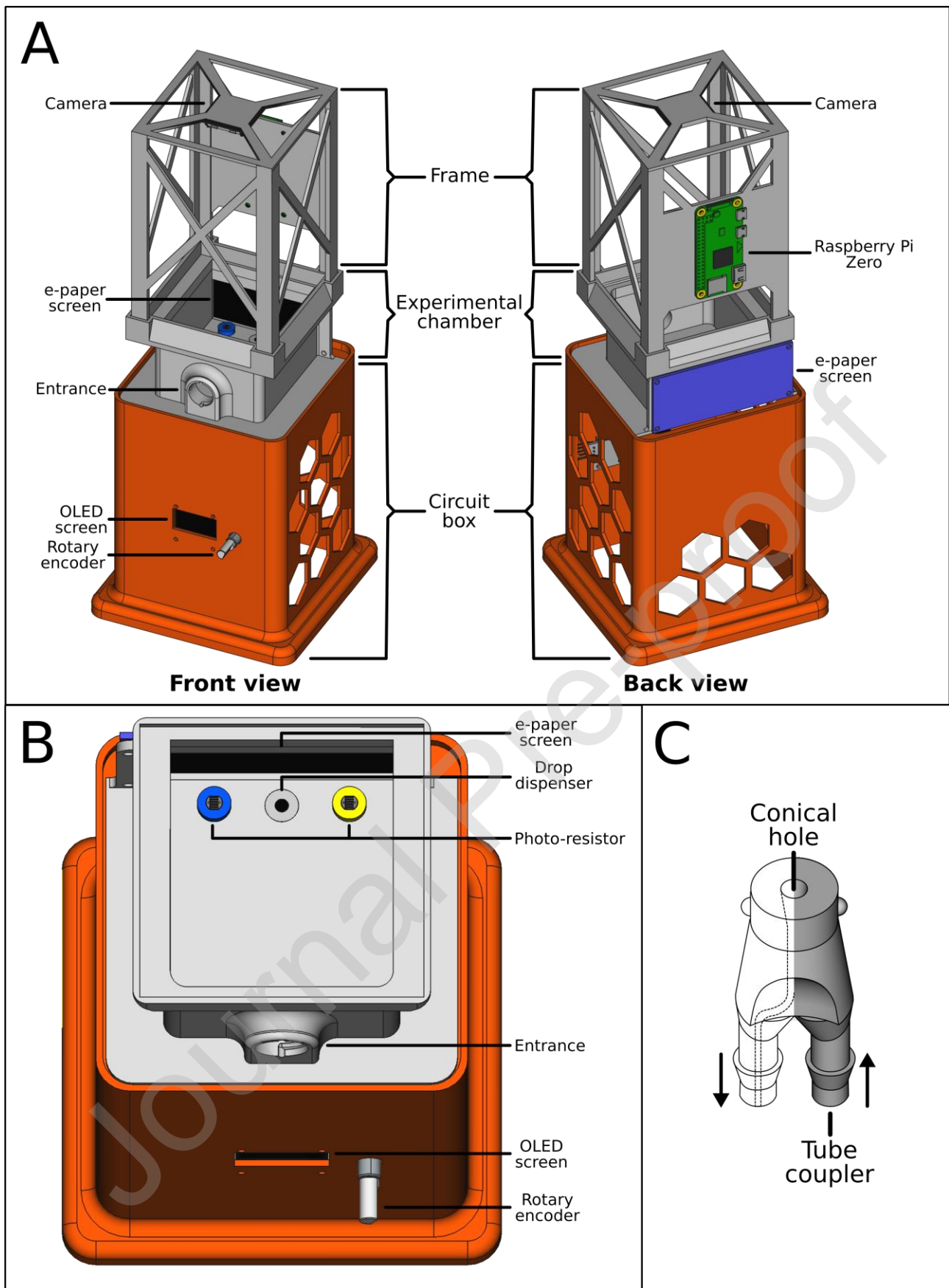


Figure 1 – The SPiDbox system. (A) Full view of the SPiDbox system, front and back view. From the bottom: (1) the circuit box, which provides space for the tubes and electrical components needed to control the experimental chamber; (2) the experimental chamber, where the training takes

place, presents an opening in the front from which the spider can be inserted; (3) a frame, on which the camera is secured, pointing downwards in order to record events happening inside the experimental chamber. The Raspberry Pi is attached to the back of this frame. (B) Top view of the SPiDbox. The frame has been removed in order to show the inside of the experimental chamber. The back wall of the experimental chamber is constituted by an e-paper screen. On the floor are fitted two photo-sensors and the drop dispenser. The two photo-sensors are respectively blue and yellow in colour. (C) Drop dispenser. On the left, the dotted line represents the internal channel for the liquid reward. The output tube of the stepper peristaltic pump gets attached to one of the two tube couplers, providing the input for the liquid (upward pointing arrow). The input tube of the DC peristaltic pump is connected to the other coupler, sucking the liquid away. The liquid reward is presented from the conical hole, that when mounted is flush to the experimental chamber floor.

Starting from the bottom, the first section is constituted by a circuit box (11cm wide, 11cm long and 15cm tall), with a window on the front to fit an OLED screen (part of the user interface, see below). Inside this box, as the name implies, all the circuits and electronic components are placed. This section also provides the needed space below the experimental chamber to fit cables and tubing that reach the components above.

The experimental chamber lies on top of the circuit box (figure 1). It is constituted by a white box (7cm wide, 8cm long and 4cm tall). On the front wall, a hole (3 cm in diameter) permits the insertion of the subject. The box floor presents three holes, positioned near the back wall. The centre hole is used to fit the reward dispenser, and the other two are used to fit the photo-sensors (see below). The back wall of the box is constituted of an e-paper screen (2.9-inch e-paper module, Waveshare) that can be used to project stimuli for the study of the animal's visual abilities. I chose to use an e-paper instead of any other alternative because it does not produce any backlighting, not disrupting the functioning of the photo-sensors and avoiding any interference with the animal's visual system (many arthropods show a phototactic response, moving towards light and possibly ignoring the task). The ceiling of the experimental box was sealed with a transparent plastic lid in order to allow videoing of the spider. On top, a second plastic frame was placed to lock the lid in place. This top frame presented all-around a 12V LED string to provide constant lighting in the box.

Lastly, on top of the experimental apparatus, a 13cm-tall frame was placed, with a pi-camera attached at the top, aimed towards the experimental box to record the experiment. The Raspberry Pi Zero was secured to the back of this frame.

In total, the three components occupy an area of 11×11×35cm. The two pumps needed to provide and remove the reward were placed outside of the box, on each side.

3.2 Reward dispenser

According to previous literature, a sucrose solution was chosen to act as a reward. It has in fact been demonstrated that many jumping spider species feed on flower nectar in nature (Jackson et al., 2001), and this reward has been reported in the literature as appropriate for training (Liedtke and Schneider, 2014). The SPiDbox employs a peristaltic pump based on a Nema 17 motor stepper motor, with 200 steps per rotation. The motor is controlled by a Polulu A4998 driver breakout board (Sparkfun). The peristaltic pump contains silicone tubing with an internal diameter of 2 mm. The peristaltic pump head has a total diameter of 3.4 cm and contains 6 rotors, each with a diameter of 0.7 cm. One end of the peristaltic pump tubing is placed inside a sucrose solution reservoir, and the other is attached through a coupler to a drop dispenser: a small plastic piece with two couplers at the bottom and a conical hole at the top. This drop dispenser is attached to the experimental box, flush with the floor. This way, when the peristaltic pump is activated, a drop of sucrose solution appears on the floor of the experimental box. After a set amount of time, the reward is removed by a second DC motor-based peristaltic pump. One end of the tubing of this peristaltic pump is attached to the drop dispenser, and the other is inserted into an empty cup, acting as a discard reservoir.

To provide the same amount of sucrose solution every time, I designed a motion routine for the stepper motor peristaltic pump. In such a pump, at different rotation angles a different amount of liquid is dispensed: at certain angles of the rotation, the rotors engage the tubing at the entrance of the pump, and successively, at a different angle, rotors disengage it at the exit. While the tube is engaged, the liquid gets pushed onwards, and when any rotor disengages the tube, the amount of liquid that it displaced is sucked back into the tubing. To successfully deliver the same amount of liquid every time, the software must know the position of the rotors at every moment. For this reason, before each experiment, the pump needs to be primed: the motor moves continuously to collect the liquid inside the tubing and send it up to the experimental box. The motor has to be stopped by the user as soon as the drop is seen coming out of the drop dispenser. After that, the motor must be rotated until it reaches a predetermined position, set as point 0 for the motor. By knowing where the 0 point is, and by keeping track of the number of steps moved from that point onwards, the machine can predict which steps will be 'push' steps and which will be 'retract' steps. After the motor reaches its starting position, the excess solution still in the drop dispenser is removed and the experiment can be started. Given the 'push-retract' pattern of the pump and the volume of liquid that the food dispenser can contain, $1/6$ of rotation is needed to fill it.

The machine can never provide multiple drops at the same time. Before being able to dispense a second, the first has to be removed by the DC motor peristaltic pump. This way, the drop dispenser can either be full or empty, never changing the amount of sucrose solution that it contains.

During experiments, the drop was dispensed only when the spider touched a particular object, acting as the unconditioned stimulus.

3.3 Data recording

In the two lateral holes of the experimental box, I fitted two different plastic pieces with a photo-resistor attached in the middle. The two objects can be of the same or of different colours, at floor level or raised, etc. The photo-resistors were subsequently attached to a voltage divider, feeding its output to a comparator circuit. The other end of the comparator was attached to the output of a potentiometer, for which the user can set a specific resistance. The photo-resistor has a variable resistance depending on the light hitting it; when the spider passed on top of the photo-resistor, it decreased the amount of light reaching the surface and increased its resistance. The comparator output switched from a 0 (photo-resistor not covered) to a 1 (photo-resistor covered) when the photo-resistor value became greater than the potentiometer one. I regulated each threshold in order to detect an activation when at least 50% of the photo-resistor surface was covered. Each activation of the photo-resistor was registered in a CSV file, along with the time of the day and duration.

The activation of one photo-resistor works as an input for the machine, which in turn dispenses a drop of sucrose solution. Having only one photo-resistor, however, would not have been enough to measure learning rate: the change in the amount of activation of just one sensor may represent an increase in the general activity of the animal, rather than a sign of learning. By inserting in the apparatus a second sensor, that however had no effect on the delivery of the reward, I could compare the number of activations between the “buttons”, enabling discrimination of a general increase or decrease in activity from the effect of a learned association.

3.3.1 Errors in the readings

From the experimental validation of the system (see next section) it appeared evident that the system implemented to detect the spiders when passing on the photo-resistor was not ideal. The comparator-based activation of the sensor is based on a fixed threshold, provided by the manual adjustment of a potentiometer. As such, it could not account for the natural fluctuations of the ambient light, forcing the experimenter to set the threshold as low as possible to avoid false positives. This however increased the number of false negatives, like sudden activation and deactivations, while the spider moved slightly or rotated its body while still remaining on top of the sensor. Because the threshold was set so low, minimal movement of the animal could cause the reading to bounce above and below the set level. Moreover, this system relied too much on human judgement, because the trimmer had to be manually set by the experimenter. The false readings could be easily identified and removed during data analysis (see next section), but an improvement to the system to prevent those from happening was still desirable.

For this purpose, I decided to design a new reading system, switching from the comparator, threshold-based digital reading (0 and 1) to an analogical one. Both the comparator and the trimmer were removed, feeding the output of the voltage divider directly to an analog-to-digital converter (ADS1116, breakout board from Adafruit). This way, the Raspberry Pi can read the exact resistance of both sensors in each reading cycle (the ADS1116 is rated for 860 cycles per second, although the delays created by the rest of the software bring it down to around 100), calculating a moving average with the following formula:

$$x = (c \cdot x) + [(1 - c) \cdot x_1]$$

where x is the calculated average, x is the reading for the current cycle, x_1 is the calculated average of the previous cycle, and c is a constant that determines the level of smoothing applied to the resulting variable. The user may want to change this variable, as noise in the photo-resistor reading and lighting inconsistencies can change between setups, although the default value should work for most people. Note that the reading x is already being smoothed, averaging the last three raw readings x_r together, in order to remove small random fluctuations. To detect an activation of the sensor, instead of using a threshold value as before, a Δ value was calculated for each raw reading, defined as the difference between x (the current read value) and x (the moving average). An activation was registered when Δ exceeded a predetermined value t . The default value t is defined such that an activation is registered when 50% of the sensor is covered. If the user wants to use different sensors, or wants to specify a different activation threshold, this constant should be changed. When an activation is detected, the moving average is locked on the last calculated value.

The activation is considered over when the value of Δ became lower than $\frac{t}{2}$, preventing unwanted deactivations due to random fluctuations of Δ around the value t . When a deactivation occurs, the moving average is then calculated again as normal.

3.4 User interface

For the machine to be used even by people not accustomed with the Python script, I designed a user interface from which the experimenter can select training routines, define subjects, and prepare experiments. I added to the box a rotary encoder, used to navigate to a menu projected onto an OLED SSD1306 (driven with the library luma.oled; Hull, 2019). At start-up, the user is prompted with two options to choose from: launch and settings.

Selecting “launch” prompts the opening of a sub-menu, containing all defined training routines to choose from. New training routines can be programmed in Python and then added to this list to be

used. After selecting any training routine, the user is prompted to input the subject ID and the trial number. During the course of any experiment, the screen shows the subject ID and trial number, as well as elapsed time and the amount of each photo-resistor's activation.

In the “settings” sub-menu, I defined some routines to test, prepare, and clean each element of the machine. The user can prime the peristaltic pump, as described above, and clean it after the machine has been used, letting clear water run through the tubing. Moreover, from this section, new subject IDs can be defined. Lastly, the photo-resistor can be tested, prompting the sensor state on the OLED screen.

3.5 Experiment presentation

With all the components interacting as described, I designed a full training procedure. At first, the animal was inserted into the experimental box, by placing it inside a syringe with the top removed, which in turn was placed in front of the entrance hole. The animal was then gently pushed inside the box and the entrance was closed. Inside, it could interact with two buttons, a rewarding and an inert one. In this experiment, I used sensors of different colours (see experimental validation). Perhaps more interestingly, the button could have been programmed to be rewarding or not depending on what stimuli were presented on the e-paper screen, such as different shapes. The subject would stay in the box for a fixed amount of time, free to press any sensor any number of times. The procedure was then repeated on subsequent days as needed. The different number of activations between sensors across trials was used to assess learning.

3.6 Experimental validation: Colour discrimination in the jumping spider *Phidippus regius*

To test the efficacy of the SPiDbox I designed a simple colour discrimination task. Note that the following experiment is not intended to be a demonstration of the presence of any ability in the jumping spider. It has already been demonstrated that jumping spiders can see a wide colour spectrum (Girard et al., 2018; Taylor et al., 2016; Vickers and Taylor, 2018; Zurek et al., 2015). The following experiment is solely intended as a demonstration that this methodology is effective in training jumping spiders. I chose the colours yellow and blue since Liedtke and Schneider (2014) already demonstrated that jumping spiders have no pre-existing preference for either colour (see also ESM1), and can indeed learn to discriminate between the two. This way, a negative result would be fully imputable to a fault in the methodology, and not to the inability of the animals to perform the task.

Since the colour discrimination task was not the focus of the experiment, in order to minimize the number of subjects needed for the experiment, I have decided to use only one of the colours (blue)

as the rewarded one: the number of subjects available would have not been sufficient to study an eventual difference in the performance between colours.

3.6.1 Subjects

I employed 30 *Phidippus regius* in the experiment. Due to subject availability, at the time of testing 2 of those were adult females, 8 were adult males and the remaining 20 were juveniles. All the spiders were born in the laboratory, from the same breeding pair. Upon emergence from an egg-sac, the animals were kept together in the same box (39×28×27cm) and fed *ad libitum* *Drosophila melanogaster* once every two days. Upon reaching the 4th instar, the spiders were separated and housed in individual transparent boxes (17×9×6cm), which contained a cardboard egg holder cut-out, to provide shelter and enrichment, as well as a florist sponge, functioning as a water and humidity source. Box size was chosen according to Carducci and Jakob (2000). All the individuals were kept at a temperature between 27 and 29 degrees Celsius, and with a light:dark cycle of 12:12 hours. Spiders were starved a week prior to starting the first trial and were never fed outside the SPiDbox until the end of the full procedure. None of the spiders that underwent the experiment had been fed with sucrose solution before the test. A total of 3 SPiDbox were used for the experiment, such that 3 spiders could be tested at the same time.

3.6.2 Procedure

The experiment lasted a total of three weeks for each individual and it was divided into two phases.

The first phase was intended as a habituation. This section had two main goals: first, I wanted to habituate the spider to a new type of food (the sucrose solution drop) and to reinforce contingencies (the box is rewarding and the drop comes from that specific spot); second, I wanted to start an association between the colour blue and a reward. Each spider was inserted into the experimental chamber through the entrance hole. Here, no photo-sensor was present: instead, the drop dispenser was programmed to activate at random time intervals, between 30 and 90 seconds. The peristaltic pump was loaded with a blue-coloured, 0.6M sucrose solution. After being dispensed, the drop remained for 30 seconds before being removed by the second pump. The spider was free to explore the apparatus and drink from the drop for a total of two hours. After this period of time, the subject was removed from the apparatus, and the latter was cleaned to start a trial for a second spider. Each spider was subjected to a single trial each day, and the procedure lasted for 5 days.

The second phase consisted of the actual training and was divided into two blocks. For the first block, I added the two photo-sensors to the experimental box. One of those was fitted into a blue casing and the other one into a yellow casing. The two photo-sensors were then placed on each side

of the drop dispenser and remained in the same position through all the phases (however, the sides were balanced between spiders). At this point, the drop was dispensed only when the spider covered more than 50% of the blue-cased photo-sensor surface. I chose the blue photo-sensor as the correct one to exploit the positive association formed with the colour blue in the habituation phase. After being dispensed, the drop (still blue-coloured 0.6M sucrose solution) remained available for 30 seconds, then was removed. Covering the yellow photo-sensor would not trigger any event, as it was added only as a control to check if the number of activations of the correct one changed (see the previous section). However, if the yellow sensor was activated during the 30 seconds of drop presence, the latter was immediately removed. This was done to avoid inadvertently training the spider in an unwanted sequence of activation combining both sensors. Each trial lasted a total of two hours, following the same schedule as the first one, and again for a total of 5 trials. The second block was identical to the first, which I decided to repeat to test for improvement between no training and 5 days of training.

I implemented a two-day pause between each block, to account for satiation.

3.6.3 Statistical analysis

Analyses were carried out with the statistical software R 3.3.3 (R Core Team, 2017). Only the main analysis is reported here, for the full script, see ESM3, for the full data see ESM2. As suggested by Forstmeier and Schielzeth (2011) I included in the models only factors that I had an a-priori reason for including. I employed a generalized linear mixed-effect model, with subject as a random effect, using the package lme4 (Bates et al., 2015) with a Poisson error structure, since the dependent variable (the number of activations) was a count data. The model was the following:

$$\begin{aligned}
 \text{Number of activations} = & \\
 & \text{sensor (blue/correct or yellow/wrong)} + \\
 & \text{test block (1 or 2)} + \\
 & \text{test number (1 to 5 of the test block)} + \\
 & \text{sensor * test block} + \\
 & \text{sensor * test number} + \\
 & \text{test block * test number} + \\
 & \text{sensor * test block * test number} + \\
 & \text{random effect (subjects)}
 \end{aligned}$$

The goodness of the fit was checked with the package DHARMA (Hartig, 2018), I had to ascertain a case of zero inflation. Accordingly, I used the package pscl (Jackman, 2017; Zeileis et al., 2008) to

perform a generalized mixed-effect model with Poisson error structure, with included a binary model to calculate for zero-inflation. Subsequently, the significance of the model predictors was calculated with an analysis of deviance carried out with the package car (Fox and Weisberg, 2011). Afterwards, a post-hoc Bonferroni corrected analysis was run on the factor that showed to have an effect on the dependent variables with the package emmeans (Lenth, 2018). Lastly, the plots were generated using the package ggplot2 (Wickham, 2009).

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4 Results and discussion

4.1 Performance and errors

Since this was the first experiment ever carried out on this system, I needed to assess its reliability and correctness. Before proceeding with the main analysis, I fully reviewed the video recording of the experiment and compared it with the automated data collection of the machine. In two out of the 300 total training trials, the system registered a total of 200 activations of the wrong sensor, whereas reviewing the videos showed clearly that the subject never touched either sensor. This error was probably due to an imprecise setting of the trimmer controlling the threshold level of the aforementioned sensor. In fact, the two trials were of two consecutive days from the same machine. The two trials were excluded from the analysis.

As anticipated in the methods section, I also observed some fast deactivation and reactivation of the sensor. Reviewing the videos showed that this happened in cases in which the spider rotated its body, maybe attracted by the dispensing of a sucrose solution drop, while remaining on top of the sensor. Before analysing the data, in order to correctly recognize these event as a single continuous activation, I merged activations that occurred less than 0.4 sec apart into a single event.

4.2 Training outcome

The results are summarized in figure 2A. From the model emerged a significant difference between sensors (correct or wrong) (GLMM analysis of deviance, chi-square = 45.297, p-value < 0.0001) and a significant different between blocks (GLMM analysis of deviance, chi-square = 12.6204, p-value = 0.0004) but no effect of the test number (GLMM analysis of deviance, chi-square = 2.4558, p-value = 0.117) nor of any of the interactions. The post-hoc analysis revealed that overall the spiders activated the rewarded sensor more often than the non-rewarded sensor (GLMM post-hoc, estimate = 0.893, SE = 0.132, z-ratio = 6.751, p-value < 0.0001) and, in general, activated sensors less, regardless of their value (correct or wrong), in the first test block over the second one (GLMM post-hoc, estimate = -0.449, SE = 0.128, z-ratio = -3.494, p-value = 0.0029). More specifically, the spiders preferred the correct sensor over the wrong sensor both in the first (GLMM post-hoc, estimate = 0.732, SE = 0.177, z-ratio = 4.127, p-value = 0.0002) and in the second block (GLMM post-hoc, estimate = 1.053, SE = 0.189, z-ratio = 5.56, p-value < 0.0001). Moreover, the spiders activated more times the correct sensor in the second block over the first block (GLMM post-hoc, estimate = 0.610, SE = 0.189, z-ratio = 3.233, p-value = 0.0074). However, there was no difference in the number of activations for the wrong sensor between blocks (GLMM post-hoc, estimate = -0.288, SE = 0.173, z-ratio = -1.669, p-value = 0.5708).

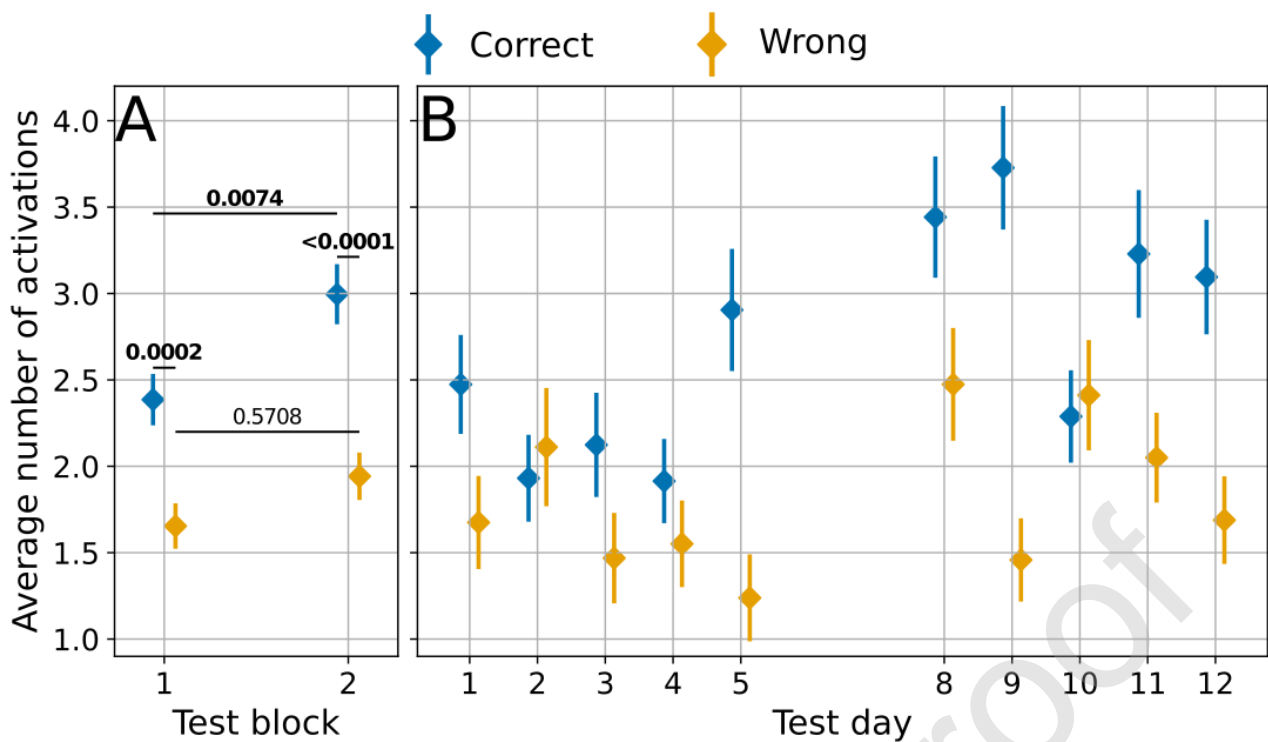


Figure 2 – **Results of the experimental validation.** (A) Here reported the average number of activation per block (dots) for each sensor (blue/correct or yellow/wrong), with standard errors. On the X-axis the two different blocks. Both in the first and in the second block the spider activated the correct sensor more than the wrong one (p value =0.0002 and <0.0001 respectively). Between block one and block two there is an increase in the amount of the correct sensor activations (p value =0.0074), while there is no increase in the wrong one (p value =0.5708). (B) Here reported the average number of activations per day for each sensor. Given the low number of subject combined with the nature of the test (see discussion) the block clustering of trials is more statistically reliable and should be trust more in term of results intrepretation. The day by day progression is shown only for completeness. Days 1 to 5 constitute block 1, days 8 to 12 constitute block 2. Days 6 and 7 are not shown, as they are the 2-day pause between the two blocks.

4.3 Discussion

The training procedure appeared to be successful since the spiders increased their number of visits towards the correct, blue sensor over test blocks while maintaining the same amount of visits to the wrong, yellow sensor. Note that I did not expect a decrease in the number of visits to the yellow sensor since it presents no negative effect on the spiders. Accordingly, the spider continued to visit, most likely randomly, the wrong sensor. On the other hand, the increase in the number of activations of the blue sensor attests that the spiders were increasingly attracted to it over test blocks, suggesting that they learned the association between the sensor and the reward.

It is worth noting that there were more visits to the correct sensor compared to the wrong sensor even in the first test block. There are two possible explanations for this effect. This early choice

could be an effect of a pre-existing innate preference of the spiders. Even though Liedtke and Schneider (2014) found no such preference, I cannot exclude this possibility, as they employed a different species of jumping spiders. To rule out this explanation, I carried out a control experiment with 10 naive spiders (for a full description, see ESM1), in which the spiders were placed in a box similar to the SPiDbox but contained two blue and two yellow cylinders, and left to explore for 2 hours. There was no significant difference in the number of contact with the blue and yellow cylinders (GLMM analysis of deviance, chi-square = 1.5904, p-value = 0.207). A significant difference may not be reasonable to expect in the control, due to the small sample size. Anyway, in the control experiment, the spiders contacted the blue cylinder 56.67% of the time, while in the first trial of the experiment they did so 64.56% of the times, representing a much higher preference over the control.

The early preference may also be an effect of the habituation phase: in fact, I used a blue-coloured sucrose solution to form an early association between the colour and the reward. If this is the case, it suggests that the spiders may be able to generalize a characteristic of the reward (the colour) to new objects. In the future, the two hypotheses about the origin of the preference in the first block could be further disentangled, replicating the experiment using yellow-coloured sucrose solution and having the yellow, not the blue, sensor as the correct one.

Because this experiment was intended only as a validation of the SPiDbox system, rather than an inquiry on jumping spiders' visual discrimination abilities, the origin of the preference in the first block is of marginal importance. Even if the spider did indeed have a pre-existing preference for the blue sensor, the increased activation between blocks clearly shows an effect of the training procedure and the formation of a sensor-reward association, as intended.

The absence of an effect of the trial may appear puzzling. In conditioning procedures a "learning curve" can generally be appreciated, describing the increase in the correctness of the subject across trials in a mostly continuous manner. The absence of this curve could be an effect of the peculiar lack of motivation that jumping spiders exhibit. As already stated in the introduction, these animals need to eat only a few times per week, a fact that makes every training procedure hard to carry out. The SPiDbox is indeed no different: the animals subjected to the experimental procedure are probably motivated for only one or two of the trials within one block, not necessarily synchronized. While spider A may be searching for food already in trial 1, spider B may be motivated only in trial 3 and randomly explored the environment in trials 1 and 2. Since it is fundamentally impossible to assess without a doubt the intent of every animal in every trial, all have to be considered. In a trial by trial analysis, no real curve can be observed within one block. The graph describing the number of contacts with the blue and the yellow sensor across trials is reported in figure 2B for

completeness. By grouping the trials, however, the random exploration can be successfully filtered out as noise by the statistical model, and the effect can be appreciated (figure 2A). I hypothesize that with a higher number of subjects, or by performing more training blocks, a learning curve will be appreciable.

4.4 Future directions and alternative usages

In the future, this system may not only be used to train jumping spiders but could be extended to many similarly sized, solitary arthropods. The consumption of sucrose solution is widespread amongst many invertebrates, and as such, I expect it to be a suitable reward for many species. However, the biology and ethology of each species should be thoroughly understood before designing the training procedure. The system could also be used for social arthropods, but it would require some modifications: The experimental box should be connected to the colony, and locomotive strategies should be taken into account (for a flying insect, a complete restructuring of the system would be needed. However, for bees and bumblebees, other alternatives already exist (Craig et al., 2014, 2012; Riva et al., 2018; Sokolowski and Abramson, 2010).

The SPiDbox is an effective system for the training of jumping spiders. Due to the easy accessibility and low cost of the components, as well as the open-source nature of the software and design, it could provide scientists with the needed instruments to study this fascinating arthropod family. As per its intended purpose, this system can be used to carry out training for complex stimuli and behaviours, increasing the number of sensors, changing their positions, inserting complex patterns of activations, and overall expanding the possibilities to study skills that were until now out of reach of scientific inquiries.

Declaration of interest

The author declares no competing interests.

Data availability

The raw data for the experimental validation are available as a supplemental material. The code, circuit schematic and 3d models of the SPiDbox are available on git-hub at <https://github.com/massimodeagro/SPiDbox>.

CRedit author statement

MD: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Roles/Writing - original draft; Writing - review & editing

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