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Title: Using Enclosed Y-Mazes to Assess Chemosensory Behavior in Reptiles

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interview Statements are read by JoVE's voiceover talent.

4. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 10
Number of Shots: 29

Introduction

1. Introductory Interview Statements

NOTE to VO talent: Please record the introduction and conclusion statements as well.

1.1. This method enables assessment of exploratory behavior in terrestrial vertebrates when presented with only chemical cues. It is adaptable to many species.

1.1.1. *2.7.2.*

1.2. Y-mazes provide insight into behavioral and ecological questions in vertebrates that enable researchers to determine the relevance of specific stimuli driving animal behavior.

1.2.1. *2.3.3, 2.3.4.*

1.3. Y-mazes are highly adaptable and widely useful to assess animal behavior, especially preference and modular behavior.

1.3.1. *2.5.1, 2.4.1.*

Introduction of Demonstrator on Camera

1.4. Demonstrating the procedure will be Gretchen Anderson from USGS.

1.4.1. INTERVIEW: JoVE's voiceover talent saying the above.

1.4.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera.

Ethics Title Card

1.5. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at the U.S. Department of Agriculture and the U.S. Geological Survey.

Protocol

2. Y-maze setup and crepuscular timing protocol for the U.S. Geological Survey (USGS) trials in collaboration with National Park Service: relatively remote testing of wild-caught Burmese pythons

2.1. Begin by placing new, clean scenting paper in the Y-maze that is long enough **[1]** to overlap at the junction of the Y and cover the entire bottom surface **[2-TXT]**. Secure the ends of the papers near the boxes and the Y-junction with masking tape **[3]**. *Videographer: This step is important!*

2.1.1. WIDE: Establishing shot of talent placing the papers.

2.1.2. Papers covering the bottom surface. **TEXT: 2 arm papers = 121.9 cm; 1 base paper = 152.4 cm**

2.1.3. Talent taping the papers.

2.2. Install the partitions to block half of the base arm **[1]**. For large scenting animals, affix a heavy object that can be easily removed and cleaned behind the barrier as a brace to prevent barrier failure **[3]**. Then, install the entrance to the opposite arm. When installing the barriers, do not rip the scenting paper **[2]**. *Videographer: This step is important!*

2.2.1. Talent installing the long partition.

2.2.2. Talent installing the short partition. NOTE: Switch the order of 2.2.2 and 2.2.3.

2.2.3. Talent affixing a heavy object behind the barrier.

2.3. Slide the acrylic top into place, one section at a time, and ensure that the angles meet completely **[1]**. Use clear plastic tape to cover any gaps **[2]**. Attach both arm boxes to the maze by connecting the faceplates with wingnuts or padlocks **[3]** and ensure that the doors are locked open **[4]**.

2.3.1. Talent sliding the acrylic top into place.

2.3.2. Talent covering gaps with tape.

2.3.3. Talent attaching arm boxes to the maze.

2.3.4. Doors locked open.

2.4. Two hours before sunset, attach the base box containing the scenting animal, keeping all movements slow and steady to minimize stress to the animal **[1]**. Arm the camera **[2]**, and open the door to the base box, latching the door in place with both barrel bolt locks **[3]**. Remain out of the animal's view and exit the area **[4]**. *Videographer: This step is difficult and important!*

2.4.1. Talent attaching the base box with the animal. NOTE: Slated as 2.4.2

2.4.2. Talent arming the camera. *Videographer: Obtain multiple usable takes of this shot, it will be reused in the results section.*

2.4.3. Talent opening the door to the base box.

2.4.4. Talent exiting the area. *Videographer: Obtain multiple usable takes of this shot, it will be reused in the results section.*

2.5. After 3 hours, note the location of the animal within the maze as well as the ambient conditions **[1]**. If the animal is in transit, wait until it enters the box **[2]**.

2.5.1. Talent making notes of the animal's location and ambient conditions.

2.5.2. Animal entering the box. *Videographer: Please attempt to film this during the shoot. If it's not possible we will use Authors' footage.*

2.6. If the animal is in any box, close and secure the box door **[1]**, remove the box, and then remove the animal, taking care to prevent defensive scent deposition in the box **[2]**.

2.6.1. Talent closing the box door.

2.6.2. Talent removing the box.

2.7. If the animal is motionless inside the body of the maze, use visual cues to stimulate its movement **[1]** into the box **[2]**. If the animal stays in place, remove the arm boxes so that the acrylic top can be removed **[3]**, then collect the animal manually **[4]** and transfer it to a bag **[5]**.

2.7.1. Talent using visual cues to stimulate the animal's movement.

2.7.2. Talent closing the box with the animal.

2.7.3. Talent removing the acrylic top and the occupied arm box.

2.7.4. Talent lifting the animal from the box.

2.7.5. Talent releasing the animal into a bag.

2.8. Partially disassemble the maze to allow removal of the interior partitions while taking care to avoid disturbing the scent paper **[1]** and then reassemble it **[2]**. If defecation occurred, collect and absorb as much as possible with clean microfiber cloths, but do not wash the area **[3]**. *Videographer: This step is difficult and important!*

2.8.1. Talent disassembling the maze and removing partitions.

2.8.2. Talent reassembling the maze.

2.8.3. Talent cleaning droppings.

2.9. Attach the base box to the base of the Y-maze using wing nuts or padlocks. Use slow, steady movements when holding and transporting the box to minimize stress to the focal animal **[1]**.

2.9.1. Talent attaching the base box to the base of the Y-maze.

2.10. Ensure that both arm box doors are latched opened **[1]**, then begin the focal trial by opening and latching the base box door using barrel bolts **[2]**. Remain out of the animal's view and exit the area **[3]**. *Videographer: This step is important!*

2.10.1. Open box arm doors.

2.10.2. Talent opening and latching the base box door.

2.10.3. Talent exiting the area.

Results

3. Results: USGS Y-maze Variables

3.1. A multitude of variables can be recorded and scored from Y-maze trials. The majority of studies using Y-mazes report simple binary choice data and analyze the results with parametric statistics such as a binomial test **[1]**.

3.1.1. LAB MEDIA: Figure 2.

3.2. A series of statistical thresholds per study sample size are depicted here, demonstrating how many successes would need to occur for a given binomial test to yield statistically significant results. These are mathematically derived and therefore generalizable to any Y-maze test **[1]**.

3.2.1. LAB MEDIA: Figure 2.

3.3. Throughout the duration of an experiment in which focal animals are observed, a variety of individual behaviors can be quantified **[1]**. Video recordings facilitate behavioral scoring. Temporal aspects of animal performance can also be quantified during Y-maze trials **[2]**.

3.3.1. LAB MEDIA: ParkerCurrylowetal-JoVE-pythonYmazevideo.mp4.

3.3.2. *Use 2.4.2.*

3.4. With any studies involving animal behavior, observer bias significantly influences data collection. Therefore, observers should be blind to the treatment being tested. The simplest way to do this is to code the video files numerically and then randomly sort them prior to assigning them to observers **[1]**.

3.4.1. *Use 2.7.4.*

Conclusion

4. Conclusion Interview Statements

4.1. Any experimental apparatus in chemoecological studies like Y-mazes must be constructed to allow low-stress, easy removal of the animal within and should be easy to disassemble for thorough cleaning and reset.

4.1.1. *2.4.1, 2.10.*

4.2. Stimuli can be tested in unique combinations to determine multimodal influence of cues, such as changing contexts of cue presentation. For example, visual and chemical cues can be presented.

4.2.1. *2.5.2.*

4.3. Although Y-mazes have been used for approximately 100 years to test reptile behavior, this approach enhances the ability to work with large and invasive species as well as to remotely capture video data.

4.3.1. *2.4.2.*