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Title: A Modified Mirror Test as A Visual Guide for the Self-awareness Trait in Wild Antarctica Penguins, *Pygoscelis adeliae*

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

√ Correct

- **2. Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **NA**
- **3. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **NA**
- **4. Proposed filming date:** To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: 06/05/2025 (mm/dd/yyyy)

When you are ready to submit your video files, please contact our Content Manager, <u>Utkarsh</u> Khare.

Current Protocol Length

Number of Steps: 06 Number of Shots: 10



Introduction

- 1.1. <u>Prabir Dastidar:</u> The scope of my research is to understand how life style, social behavior and repetitive activities of penguins in a rookery are imprinted in the memory system which, in turn, reflects in their response to the mirror images. Authors: Edited the statement for clarity and making it in a statement, full sentence style.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

What are the most recent developments in your field of research?

- 1.2. <u>Prabir Dastidar:</u> As such, apparently, there is no work reported on the self-awareness studies on penguins using modified mirror test. It is first time, experiment is done on self-awareness in its natural habitat.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

What significant findings have you established in your field?

- 1.3. <u>Prabir Dastidar:</u> Day to day activities, life styles contribute to the formation of atomic habits. Penguins who live in a homogeneous group develop an intrinsic system of mindset. Their reactions to the mirror images are reflections of their mental framework.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 6*

How will your findings advance research in your field?

- 1.4. Prabir Dastidar: Our findings will initiate more research on the structure and functioning of primitive brain having no pre-frontal cortex with organized behavior of animals. How minimal mental computations lead to an orderly and organized behavioral regime. Authors: Edited the statement for clarity and making it in a statement, full sentence style.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 8*

What research questions will your laboratory focus on in the future?

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- 1.5. <u>Prabir Ghosh Dastidar:</u> In the future, my laboratory will focus on investigating the role of primitive brain in defining the intrinsic mental conditions and its relations with the cooperative behavior and functioning of a homogeneous social group of animals.

 Authors: Edited the statement for clarity and making it in a statement, full sentence style.
 - 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Ethics Title Card

This research has been conducted in Antarctica with strict adherence to the procedures and protocols outlined in ATCM Resolution 4 (2019) and Annex II of the protocol on Environmental Protection to the Antarctic Treaty



Protocol

Video editor: The author provided footage later and no timestamps were requested as the author was difficult to deal with and postshoot note integrator (Swati) added representative timestamps after she looked through the footage as suggestions! Please feel free to select the patch that is most stable for video purposes. Also, please go through all footage once and if you find a better video for the same visuals, please use that.

2. Penguin Behavior Studies in Closed Enclosure

Demonstrator: Prabir Dastidar

- 2.1. To begin, select a plain patch of land on the rough terrain of the selected site in Antarctica for conducting experiments [1]. Set up the experiment near the rookery where the nests of the penguins are located [2].
 - 2.1.1. LAB MEDIA: VIDEO 1 Familiarisation with penguins in Dogs Neck ice-shelf.movh265: 02:06 – 02:20
 - 2.1.2. LAB MEDIA: Figure 1 B or VIDEO 10 Long view of the Penguin colony at Svinner island.mov-h265
- 2.2. Place a large, securely supported mirror on the ground in the path of Adelie penguin waddles. Closely observe their reactions to their reflections and keep the mirror in position as long as the penguins remain attracted to it [1-TXT].
 - 2.2.1. LAB MEDIA: Figure 2 TXT: Record the stay time
- 2.3. For the modified mirror test, construct two three-sided, closed enclosures by fixing cardboard sheets [1-TXT]. Use the bigger enclosure for the main experiment [2] and the small enclosure to close or expand the larger enclosure [3].
 - 2.3.1. LAB MEDIA: Figure 3 A or VIDEO 2 Making of the experimental set up..3526.1.36..mov-h265 **TXT: Dimensions: 75 cm x 100 cm and 75 cm x 50 cm**
 - 2.3.2. LAB MEDIA: Figure 3 A Video editor: Please emphasize the C-shaped cardboard in the top half of the image.
 - 2.3.3. LAB MEDIA: Figure 3 A Video editor: Please emphasize the V-shaped cardboard in the bottom half of the image.
- **2.4.** After observing the behavior in a large enclosure without a mirror, place two glass mirrors on the opposite sides of the larger enclosure to ensure that the penguins

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confront their mirror images within the enclosure and take photographs before releasing them [1].

- 2.4.1. LAB MEDIA: VIDEO 3 MMT1 and color-bib.mov-h265. *Video editor: Between first 2 minutes, select any range where both mirrors are seen and penguin is moving between them and interacting with mirror.*
- 2.5. Prepare and fasten a brightly colored bib around the penguin's neck, ensuring the bib does not obscure the body contour. Record the durations in the enclosure with and without a bib and capture images [1].
 - 2.5.1. LAB MEDIA: VIDEO 7 MMT & C Bib Test.mov-h265
- 2.6. For the hidden head test, attach a colored paper disk on the front mirror to block penguins' head and upper body views. Document penguins' durations with and without disk and capture images [1].
 - 2.6.1. LAB MEDIA: VIDEO 6 MMT,HHT and C Bib.mov-h265: 02:10 02:40 **TXT: Disc** diameter: **14** cm



Results

3. Representative Results

- 3.1. In the modified mirror test, penguins consistently spent more time in front of their mirror images than during the control phase [1]. They frequently made rapid, sometimes repeated head and flipper movements, performed various body gestures, and occasionally switched sides [2]. None appeared restless or fearful, and all maintained constant visual attention to their mirror images [3].
 - 3.1.1. LAB MEDIA: Table 1 Video editor: Please emphasize Average and Median rows.
 - 3.1.2. LAB MEDIA: Figure 9 *Video editor: Please show 1st and 3rd images*
 - 3.1.3. LAB MEDIA: Figure 9 *Video editor: Please show 2nd image and crop to remove the area where green paper is visible*
- 3.2. In the hidden-head test, a stark behavioral difference was observed during the experimental phase when their head and upper body reflections were obstructed [1], compared to the unobstructed control phase [2].
 - 3.2.1. LAB MEDIA: Table 2 Video editor: Please emphasize the "Duration of stay during experimental phase (With sticker on mirror)" column
 - 3.2.2. LAB MEDIA: Figure 2 Video editor: Please emphasize "Duration of stay during control phase" column
- 3.3. In the colored-bib test, behavior remained normal after wearing the colored bib. Despite the longer duration, no signs of distress or discomfort were observed [1].
 - 3.3.1. LAB MEDIA: Table 3 *Video editor: Please emphasize the rows with serial numbers 3, 5, and 6.*