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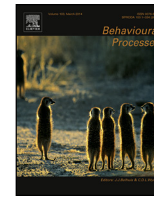

Light and shadow: Visual recognition of the stationary environment by leopard frogs

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ABSTRACT

We determined how leopard frogs respond to non-moving aspects of the environment. We have discovered that these frogs are attracted to dark, stationary, opaque objects. This attraction depends on the relative reflectance of the object, i.e., the darker the block, the more attractive it is, and the attraction is found under both bright and dim ambient light levels. Larger blocks are more attractive than smaller blocks, but frogs are still attracted to blocks much smaller than themselves. Previous studies have shown that frogs are also attracted to sources of light. Using a choice experiment, we show that the probability a frog will choose a dark object versus a light source depends on the intensity of the light source relative to the intensity of the ambient light. The frog only moves toward a light source when it is at least 20 times brighter than the brightest object in the environment. These findings help to clarify the frog's "phototactic" nature.

1. Introduction

Leopard frogs rely on vision to catch moving prey and to avoid looming predators. There are some who suggest that frogs are "not concerned with the stationary parts of the world" (Lettvin et al., 1959) or that frogs cannot see stationary objects (Kandel et al., 1991). Nonetheless, frogs are able to navigate around stationary obstacles in their environment, which they usually jump around or over. However, we have discovered that when an opaque obstacle is quite dark (i.e., low relative reflectance), a frog will spontaneously jump toward it and even collide with it. Frogs are attracted to black objects and frogs are also attracted to dark holes and shadows. In addition, previous research dating back to the 19th century showed that frogs are attracted to light sources (Cole, 1907; Fite et al., 1978; Kicliter, 1973; Muntz, 1962; Parker, 1903; Pearse, 1910; Plateau, 1889; Ralph, 1978; Torelle, 1903). We hypothesize that frogs move toward a light source or a dark area depending on contingencies of the environment.

The question of how frogs respond to light and dark areas is part of a larger question: how do frogs recognize their stationary environment? In order to be seen, images of objects projected onto the retina need to be in motion. Humans are able to see stationary objects because the images of those objects are kept in motion on the retina through microsaccades (Yarbus, 1967). Frogs keep images of the stationary world in motion relative to the retina through periodic head movements associated with breathing (Skorina et al., 2011). In this paper we challenge frogs in novel environments consisting of dark and light areas to elucidate how frogs recognize aspects of their stationary environment.

2. Materials and methods

2.1. Setup

Adult leopard frogs (*Rana pipiens*), 6–7.5 cm snout to vent were obtained from Vermont (Hazen Company, Alburg, VT, USA), housed in a terrarium (32 cm × 76 cm × 31 cm, $H \times W \times D$) with running water, kept on a 12 h light/dark cycle, maintained at 21–23 °C, and fed live crickets (*Acheta domestica*) 3 times a week. Frogs, drawn from a pool of 12 animals, were tested in a 60 cm × 60 cm ($H \times W$) arena made of 60 cm high white walls (80% relative reflectance) with a white floor (80% relative reflectance). A white umbrella (104 cm diameter) was everted and hung upside down so that its

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edge was 34.5 cm above the arena. The arena was uniformly illuminated indirectly by two white compact fluorescent lights (19 W, Ecosmart) placed on opposite sides outside the arena, directed up toward the umbrella and shielded by the 60 cm high walls of the arena. Incident light levels were measured using an ORIEL Light Meter Model 70286. The incident light level of the standard arena was 2.3 μW and was measured by placing the light meter in the middle of the arena, 5 cm above the ground, and directing the sensor upwards. Stimuli consisted of 1.8 cm thick rectangular wood blocks of various dimensions. To determine the relative reflectances of the blocks, which were of various shades, we began by sampling the power of light reflected off a standard gray card (18% reflectivity, Digital Image Flow). Under identical incident light conditions we measured the intensity of light reflected off the various blocks. We then calculated the relative reflectances of the blocks in relation to the known reflectivity of the gray card.

2.2. Statistical analyses

To evaluate the statistical significance of our results, we used The R Project for Statistical Computing version 3.0.1 for performing binomial and multinomial logistic regressions, and MatLab R2013B for performing a *Z*-test.

2.3. Scoring protocol

We tested frogs' responses to opaque blocks placed 15–30 cm away. Behavior was characterized as: *Attraction*: if the frog moved at least halfway toward the block; *Other Movement*: if the frog moved away from the block; *Null*: if the frog failed to move in 1.5 min. Between trials the frog was taken out of the arena and placed in a 10 cm × 19 cm × 27 cm ($H \times W \times D$) transparent plastic box left for at least 1 min.

Frogs were placed in the test arena and tested immediately. In test sessions we usually alternated between testing of two frogs. The test objects used as stimuli were already in place in the arena. If a frog did not respond within 1.5 min the test session was terminated until the next day.

2.4. Attraction to blocks of different relative reflectance

We tested frogs' responses to one of five blocks, each of a different relative reflectance. The blocks were all 14 cm × 8 cm ($H \times W$) in size and consisted of five shades: *black*, 6% relative reflectance; *dark gray*, 13% relative reflectance; *intermediate gray*, 24% relative reflectance; *light gray*, 41% relative reflectance; *white*, 81% relative reflectance. A randomized order was used in choosing from the five-block set. One block was placed against an arena wall equidistant from the vertical edges of that wall. Each frog ($N = 5$) was placed in the center of the arena directly facing the block, with its snout 30 cm away from the block. Each block was presented a total of 15 times to each frog. The data were analyzed using a binomial logistic regression. Relative reflectance was treated as a continuous independent variable and the response was the proportion of the trials the frog was attracted to the block.

2.5. Contrast between block and wall

We tested frogs' responses to three blocks of different relative reflectances (black, dark gray, and white as described in Section 2.4) placed against walls of different relative reflectances. The three wall reflectances were: *black*, 6% relative reflectance; *gray*, 27% relative reflectance; *white*, 80% relative reflectance. Different combinations were used: black block/white wall; dark gray block/white wall; black block/gray wall; white block/gray wall; dark gray block/black wall. For each trial, a block was placed against an arena wall equidistant from the vertical edges of that wall. Each frog ($N = 5$) was placed in the center of the arena directly facing the block, with its snout 30 cm away from a block. Each block/wall combination was presented 15 times to each frog. For each background wall the order of presentation of the blocks was randomized.

To determine if there is a behavioral difference between a lighter block on a darker background and a darker block on a lighter background, we performed a multinomial logistic regression with the darker object as a factor.

The contrast ratio between the block and background wall is defined as:

$$\text{contrast ratio} = \frac{|\text{reflectance of block-reflectance of wall}|}{\text{reflectance of brighter object}}$$

where reflectance = amount of power sampled off of the object (block or wall).

2.6. Choice between blocks of different relative reflectance

We tested frogs' choices between blocks (14 cm × 8 cm) of different relative reflectance: intermediate gray versus black; dark gray versus black; intermediate gray versus dark gray. The two blocks were set against an arena wall 18 cm away from the vertical edges of that wall, leaving an 8 cm gap between blocks. Each frog ($N = 5$) was placed 15 cm away from the wall, facing the center of the gap. For each pair of blocks, the left/right position of the blocks was randomized. We used a different scoring protocol than that listed in Section 2.3: we noted if the frog moved to within 7 cm of one of the blocks, or moved away from both blocks, or did not move at all in 1.5 min. The frog was placed in a 10 cm × 19 cm × 27 cm ($H \times W \times D$) transparent plastic box for at least 1 min between trials. For each frog, trials continued until it had moved toward the blocks a total of 20 times. Data were analyzed using a 2-tailed, 1 sample *Z*-test.

2.7. Attraction to blocks of different sizes

We tested frogs' attraction to black blocks of approximately the same relative proportions (ratio of height to width ≈ 1.75) but different sizes. The dimensions of the blocks ($H \times W$) were: 14 cm × 8 cm; 10 cm × 5.6 cm; 7 cm × 4 cm; 5 cm × 2.8 cm; 3.5 cm × 2 cm; 2.5 cm × 1.4 cm; 1.75 cm × 1 cm; 1.25 cm × 0.7 cm. A block was drawn at random from the set and placed against an arena wall equidistant from the vertical edges of that wall. Each frog ($N = 5$) was placed in the center of the arena directly facing the block, with its snout 30 cm away from the block. Each block was presented a total of 15 times to each frog. We performed a binomial logistic regression to analyze the effect of the size of the block on frogs' attraction, with the natural logarithm of the surface area serving as a continuous independent variable.

2.8. Attraction to blocks of different relative reflectance under dim incident light

We tested frogs' ($N = 5$) responses to blocks of different relative reflectance in dim incident light intensity (0.023 μW, whereas the standard incident light intensity was 2.3 μW). Trials were conducted as in Section 2.4. Black, intermediate gray, and white blocks were presented. The results were analyzed with a binomial logistic regression model using relative reflectance of the blocks as one continuous independent variable.