

# Slope-Based and Geometric Encoding of a Goal Location by the Terrestrial Toad (*Rhinella arenarum*)

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The current study was designed to test for the ability of terrestrial toads, *Rhinella arenarum*, to use slope as source of spatial information to locate a goal, and investigate the relative importance of slope and geometric information for goal localization. Toads were trained to locate a single, water-reward goal location in a corner of a rectangular arena placed on an incline. Once the toads learned the task, 3 types of probe trials were carried out to determine the relative use of slope and geometric information for goal localization. The probe trials revealed that the toads were able to independently use slope, and as previously reported, geometry to locate the goal. However, the boundary geometry of the experimental arena was found to be preferentially used by the toads when geometric and slope information were set in conflict.

**Keywords:** amphibians, spatial cognition, vertical navigation, slope, boundary geometry

Despite their transformative position in vertebrate evolution, representing the first transition from water to land and its consequent implications for brain evolution, extant amphibians are an underrepresented group in research on vertebrate comparative cognition (Muzio, 2013). However, during the last two decades some advances have been made understanding amphibian cognition (e.g., numerosity, see, Stancher, Rugani, Regolin, & Vallortigara, 2015; functional lateralization, see Bisazza, Cantalupo, Robins, Rogers, & Vallortigara, 1996; Lippolis, Bisazza, Rogers, & Vallortigara, 2002; Vallortigara, Rogers, Bisazza, Lippolis, & Robins, 1998). But, given the importance of spatial cognition in conver-

sations on comparative cognition, it is perhaps surprising how little we know about how amphibians represent space (for some examples of amphibian spatial cognitive ability see Daneri, Casanave, & Muzio, 2011; Sinsch, 1990, 2006; Sotelo, Bingman, & Muzio, 2015).

From an evolutionary perspective, amphibians represent the closest relatives to the amphibious tetrapods that invaded land some 350 million years ago. It is also noteworthy that the anatomical organization of the amphibian dorsomedial forebrain/hippocampal formation is less complex than in amniotes (e.g., Roth, Laberge, Mühlenbrock-Lenter, & Grunwald, 2007). However, as noted above, amphibian cognition has not gone completely unstudied and has included experiments carried out in the terrestrial toad, *Rhinella arenarum*, demonstrating considerable learning ability in this species (e.g., Daneri, Papini, & Muzio, 2007; Muzio et al., 2011; Puddington, Papini, & Muzio, 2013). Like other toad species (e.g., see Sinsch, 1987), *R. arenarum* also carries out seasonal migrations to often remote breeding ponds (Gallardo, 1974), implying a well-developed navigational ability based on some kind of learning.

A modest number of research studies done with amphibians has focused on their spatial and navigational behavior (e.g., Daneri et al., 2011; Daneri, Casanave, & Muzio, 2015; Dole, 1968; Ferguson, 1971; Fischer et al., 2001; Grant, Anderson, & Twitty, 1968; Liu, Day, Summers & Burmeister, 2016; Pašukonis, Loretto, Landler, Ringler, & Hödl, 2014, Pašukonis, Warrington, Ringler, & Hödl, 2014, Pašukonis et al., 2016; Phillips, Adler, & Borland, 1995; Sinsch, 1990). Recently, we carried out an experiment in the terrestrial toad showing that they could locate a goal in a rectangular arena relying on both the boundary geometry of the arena and a prominent feature cue on one of the arena walls (Sotelo et al., 2015). Conflict probe trials further revealed that boundary geom-

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This article was published Online First August 28, 2017.

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This research was funded in part by Grant PICT 2243 (FONCYT), by Grant PIP 0893 (CONICET), and by Grant UBACYT P0293BA (Universidad de Buenos Aires), Argentina, all to RNM. VPB was funded by NSF Grant IOS-1457304 during the carrying out of the study.

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etry had more control over the behavior of the animals despite being associated with more uncertainty regarding a goal location compared with the feature cue. As such, the importance of boundary geometry was generally consistent with what has been found in many other vertebrate groups (e.g., fish: López, Broglio, Rodríguez, Thinus-Blanc, & Salas, 1999; López, Bingman, Rodríguez, Gómez, & Salas, 2000; Sovrano, Bisazza, & Vallortigara, 2003; Vargas, López, Salas, & Thinus-Blanc, 2004a; chicks: Vallortigara, Zanforlin, & Pasti, 1990; pigeons: Kelly, Spetch, & Heth, 1998; Vargas, Petruso, & Bingman, 2004b; humans: Hermer & Spelke, 1994).

Beginning with the watershed experiment of Cheng (1986), experiments that contrast the use of boundary geometry with feature cues have been a reliable paradigm in the comparative study of spatial cognition and underlying neural substrates. However, boundary geometry and visual features are not the only potential sources of information a vertebrate can rely on to recognize a goal location. For example, terrain slope has been shown to be used by homing pigeons, rats, and humans to locate a goal (Cheng, Huttenlocher, & Newcombe, 2013; Grobety & Schenk, 1992b; Holmes, Nardi, Newcombe, & Weisberg, 2015; Nardi & Bingman, 2009a; Nardi, Funk, Newcombe, & Shipley, 2009; Nardi, Nitsch, & Bingman, 2010; Nardi, Newcombe, & Shipley, 2011, 2013; Nardi, Holmes, Newcombe, & Weisberg, 2015; Weisberg, Nardi, Newcombe, & Shipley, 2014). More generally, locating a goal in the vertical plane has been observed in fish and rats in a context different from slope (fish: Holbrook & Burt de Perera, 2009; rats: Grobety & Schenk, 1992a; Jeffery, Jovalekic, Verriotis, & Hayman, 2013; Jeffery, Wilson, Casali, & Hayman, 2015). Further, studies in rats and a fish (the sighted banded tetra *Astyanax fasciatus*) demonstrated that they can discriminate information from the horizontal and vertical axes of space and, in the case of the banded tetra fish, they rely more on vertical-component information under conflict (Holbrook & Burt de Perera, 2009; Jovalekic et al., 2011). Nardi et al. (2013, 2015) have argued that slope is potentially important as a spatial cue because of its gradient properties, providing information along an “uphill-downhill” axis. When slope is coupled with “sense” information (i.e., left or right) specific locations in an experimental space can be discriminated. Another interesting property of slope is that it can be perceived via multiple sensory modalities including vision, vestibular, and kinesthetic input.

Our objective in the current study is to build on our previous work with terrestrial toads (Sotelo et al., 2015) by investigating the relationship between boundary geometry and environmental slope in supporting goal recognition. Similar to the previous geometry-feature study, toads were found to be able to learn to rely on both boundary geometry and slope to locate a goal, and when the sources of spatial information were set in conflict, they again preferentially relied on geometry.

## Method

### Subjects

Twelve sexually mature, experimentally naive terrestrial toads (*R. arenarum*), a species not listed as threatened (IUCN, 2014), were used. The experimental subjects were captured in ponds around Buenos Aires, Argentina, during October 2014. Animals

were maintained according to the guidelines outlined by the National Institutes of Health Guide for Care and Use of Laboratory Animals (National Research Council, 2011). They were treated with antibiotics (Baytril 0.5 mg/animal  $\times$  7 doses) and anthelmintics (Mebutar 0.5 mg/animal  $\times$  1 dose) to avoid bacterial and parasitic infection, and kept in group cages with running water during the first month in the laboratory. Toads were fed once a week until 1 week before pretraining started. Standard weights (weight of the hydrated animal with its urinary bladder empty; Ruibal, 1962) were obtained the day before pretraining. Weights varied between 69.90 and 148.20 g ( $M = 108.03$  g,  $SE \pm 6.85$ ). The vivarium was kept at a constant temperature (24–27 °C) and humidity (48–52% RH), and subjected to 16:8 hr light/dark cycle (lights on at 6:00 a.m. local time).

Toads were subjected to experimental training between 2:00 and 7:00 p.m. Just before the beginning of the experiment (pretraining), animals were transferred to individual cages and dehydrated to 80% of their standard weights. This procedure successfully results in toads motivated to search for water as a primary reinforcer (Muzio, Segura, & Papini, 1992; Muzio et al., 2011).

### Training Environment

The experimental arena (see Figure 1) was the same used in Sotelo et al. (2015). Briefly, it consisted of an elevated, rectangular-shaped, white Plexiglas arena (90 cm long  $\times$  45 cm wide  $\times$  60 cm high) surrounded by a circular white curtain. For the current experiment, the arena was lifted on one of the short sides forming a 5° slope-angle with respect to the floor. The incline resulted in polarizing the two short ends of the floor, with one “uphill” and one “downhill” similar to what was used in previous homing pigeon studies (Nardi & Bingman, 2009a; Nardi et al., 2010). However, it should be noted that in the pigeon studies slope was set at 20° (in comparable studies with humans, slope has been set at 5°; Nardi et al., 2011, 2013). Four plastic, green water containers (13 cm long  $\times$  10 cm wide  $\times$  3 cm high), which were covered with a wire mesh and filled with deionized water, were placed at each corner of the rectangular arena. The water containers were aligned along the arena’s slope. Access to the water, used as reward, was enabled by adjusting the water level relative to the wire mesh. When the water level reached the surface of the wire mesh, a toad’s ventral skin surface could make contact with the water (accessible water). However, when the water level was below the wire mesh, the toad could not make contact with the water surface (inaccessible water). It should be noted that the toads could not visually recognize the water level of the containers from distance because the top of the containers was too high to gain visual access to the water inside (see Daneri et al., 2011, 2015; Muzio et al., 1992; Sotelo et al., 2015). Therefore, the procedure ensured similar, remote sensory access to the water for both the reinforcing and nonreinforcing containers. During training sessions (but not probe trials, see below), only one of the water containers was filled up to a level accessible by touch to the animals.

The arena was illuminated by a white 60 W incandescent light bulb at a height of 120 cm above the center of the arena. The animals’ movements within the arena were observed from behind one of two slots cut into opposite sides of the surrounding curtain. An inverted, opaque plastic container (10 cm long  $\times$  10 cm

wide  $\times$  8 cm high) placed in the center of the arena served to release the animals at the beginning of a trial. A constant white noise (20–30,000 Hz) was played during training and testing sessions.

## Behavioral Procedures

The experimental training used followed the same procedures as those described in Sotelo et al. (2015). At the beginning of each trial, every toad was covered by a cloth to prevent visual access during the approach to the experimental arena and placed under the start container. It remained under the start container for 30 s until the container was raised by hand, releasing the toad to move freely in the inclined, rectangular arena. Although the animals were not rotated before each trial, for each trial they were placed into the arena from different positions and oriented in different directions in the start container. This treatment should have effectively minimized the potential use of path integration to locate the goal. Each toad was trained for its once daily session of three trials before the next animal was trained. Water uptake (or weight variation) was also recorded by subtracting the weight of each toad before a session from its weight after the session. This variation was then divided by the animal's standard weight and multiplied by 100 to provide a relative measure of water uptake for each session adjusted by body weight (Daneri et al., 2011; Muzio et al., 1992). After each training session, as a consequence of water uptake during trials, the weight of the toads generally increased above 80% of their standard weight. Therefore, before the next session, they were dehydrated again until they reached the target weight.

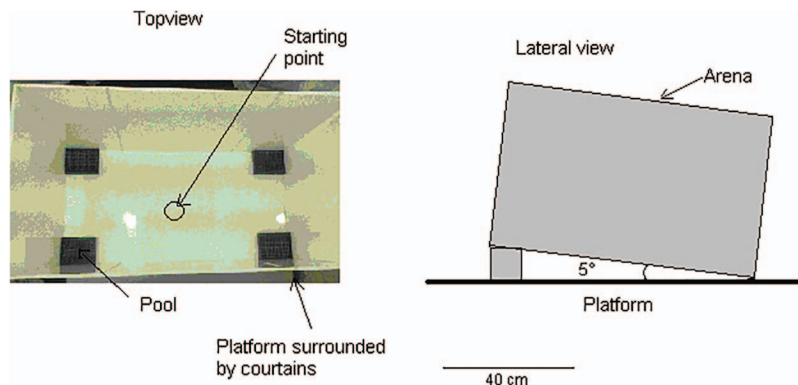
**Pretraining.** Before beginning training trials, the animals were individually pre-exposed to the rectangular arena twice (one session per day). During pretraining sessions, water was accessible at all four water containers and animals were allowed to hydrate for 10 min.

**Training.** Training began the following day (one daily session of three trials) and continued until the animals reached the acquisition criterion of three consecutive sessions with an average collective/group performance of at least 50% correct, first-choice responses. The ultimate goal of the training was to determine the extent to which boundary geometry and slope information was used by the toads to locate the goal container with accessible

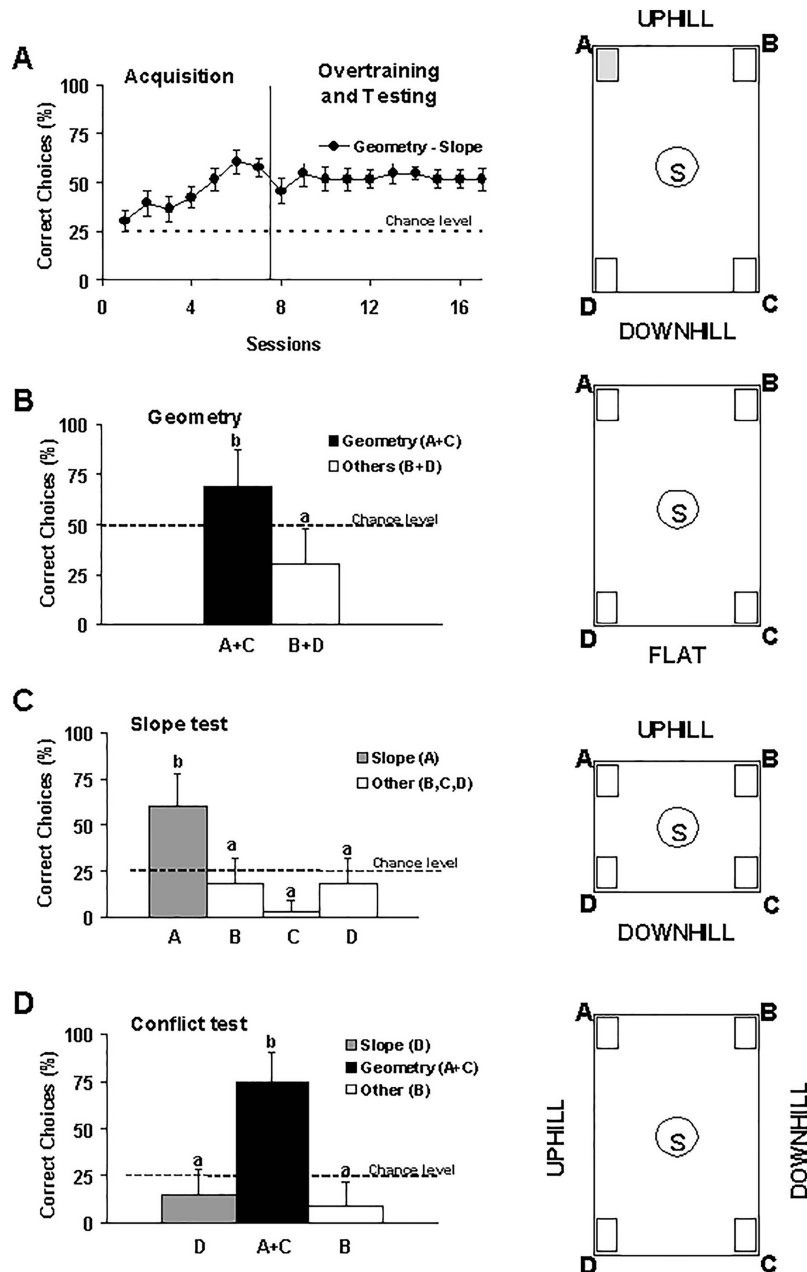
water. Noteworthy is that while geometry could be used to identify *two* candidate goal locations, only slope, coupled with sense information, for example, uphill to the left, could be used to uniquely identify the one goal container (Figure 2A, right). To control for any cues other than the boundary geometry and the slope of the arena, from trial to trial the uphill side of the arena was pseudorandomly shifted between the two short walls (the side was randomly changed either on the second or the third trial of each session for each animal). Also from trial to trial, the rectangular arena was rotated pseudorandomly clockwise or counterclockwise by 90°, with each session having one clockwise rotation and one counterclockwise rotation.

As indicated above, toads were trained until they reached as a group the acquisition criterion of a collective average of at least 50% correct first-choice responses for three consecutive sessions (use of slope and sense would result in a chance performance of 25% correct). In an experiment of this type, one has to choose whether each animal is tested to a particular criterion or if all animals receive the same number of training trials. Our intent using a group criterion was to try and be sensitive to both issues; we have a collective performance criterion while insuring all animals receive the same number of training trials. Validating this compromise approach, a similar “group criterion” has been successfully used by the Muzio laboratory in previous studies to demonstrate learning in toads (e.g., Daneri et al., 2011; Sotelo et al., 2015).

Across subjects, the goal location was balanced among the four corners of the arena. As such, 6 animals were trained with the goal uphill and 6 animals were trained with the goal downhill. Goal location was also balanced with respect to being located to the left or the right when facing the target wall. A water-container choice/trial was recorded as correct or incorrect when a toad's whole body had completely entered one of the containers. If the animal selected its correct container, it was allowed to stay for 2 min. If the choice was incorrect, it spent 1 min in the incorrect container as a penalty; afterward, it was gently guided to the correct container (guided correction trial). Each animal was tested for 3 trials per session; intertrial intervals were spent in the home cage and lasted approximately 2 min. After each trial, the arena was cleaned and the water containers were shifted among the corners as well.



*Figure 1.* Picture (top view, left) and schematic (lateral view, right) of the rectangular arena used to train toads. Four plastic green water containers were placed at each corner; only one corner was rewarded with deionized water (there were no visual cues on the walls). See the online article for the color version of this figure.



*Figure 2.* (A) Percentage of correct choices across the 17 sessions of training trials. On the right is a diagram of the experimental conditions, showing the start container (S), slope orientation, and the location of the goal (in light gray). Schematic is shown with the reinforced water container on the left face of the uphill short wall. However, the actual reinforced water container positions were counterbalanced across toads. (B) Percentage choice distribution and schematic representation of the Geometry test. (C) Percentage choice distribution and schematic representation of the Slope test. (D) Percentage choice distribution and schematic representation of the Conflict/Dissociation test. Means and confidence intervals ( $\alpha = .05$ ) are plotted. Different letters denote significant choice-percentage differences between locations.

**Probe test trials.** After reaching criterion, toads were subjected to three different probe-trial types: (a) Geometry Test; (b) Slope Test; and (c) Dissociation/Conflict Test (see schematics in Figure 2B–D, respectively). Each animal was tested three times for each probe-trial type, and on probe trials none of the four containers had accessible

water (no reward given). During probe sessions, one probe trial was inserted within a group of three training trials as either the second or third of the 4-trial probe session. Probe-trial type was pseudorandomized across sessions with the constraint that each probe-trial type had to occur once within each cycle of three sessions.

For the Geometry Test (Figure 2B, right), the slope of the arena was removed rendering the entire arena floor flat. The purpose of the Geometry-only Test was to determine whether the animals learned to use the boundary geometry of the arena to locate the goal.

For the Slope Test (Figure 2C, right), the rectangular arena was adapted to form a square-shaped arena (45 cm long  $\times$  45 cm wide  $\times$  60 cm high) creating an environment where all four corners were characterized by the same geometric properties. The square shape was achieved by adding two panels, built of the same white Plexiglas as the arena walls, inside the rectangular arena. Across trials, slope was pseudorandomly oriented (the uphill or downhill wall could be any of the added panels). By rendering geometric information irrelevant, the purpose of this test was to determine whether the toads learned to locate the goal location by means of the slope information alone.

For the Dissociation/Conflict Test (Figure 2D, right), the long walls of the arena occupied the uphill and downhill positions (still at an angle of 5°). This change resulted in one corner of the arena being correct with respect to slope (e.g., the goal is located to the left of the new uphill), two corners being correct with respect to boundary geometry and one corner that could not be the goal corner based on either slope or geometry. The purpose of the Dissociation/Conflict Test was to determine whether slope or geometry was more potent in controlling the behavior of the animals when the sources of information were put in conflict (i.e., they provided contradictory information with respect to goal location).

## Statistics

We used the statistics program Infostat (Universidad Nacional de Córdoba-FCA-UNC-, Argentina) to analyze the data. Statistical comparisons were carried out using one way analysis of variances (ANOVAs) and post hoc least significance difference (LSD) tests. In one case, the assumption of normality and homoscedasticity was violated ( $p < .05$ ) and the nonparametric Kruskal-Wallis test was substituted. Friedman tests were performed using the software SPSS (IBM SPSS Statistics 20.0) to analyze differences from chance levels. Effect sizes for every test ( $\eta_p^2$ ) were also calculated using SPSS.

## Results

### Training Trials

Figure 2A (left) shows the mean percentage of correct choices to the goal location during the training trials. Across training (acquisition) sessions, toads increased choice accuracy, reaching criterion on Session 7 after collectively meeting or exceeding 50% correct choices from Sessions 5–7. Performance across the last three training sessions was higher than that expected by chance (Friedman,  $\chi^2(3, N = 11) = 19.69, p < .001$ ). Performance levels on training trials remained steady during the postcriterion, overtraining and testing sessions. Finally, it is noteworthy that the toads of the current study, trained with both slope and geometric information, learned the location of the goal considerably faster when compared with toads trained with geometry-only or geometry together with a polarizing visual feature cue (Sotelo et al., 2015; see Discussion).

### Test Trials

Figure 2B (left) shows the toads' performance on Geometry test trials, when arena slope information was removed. No differences were found with respect to choices between the two possible correct corners (only geometry could be used for this test-trial type;  $p > .99, F = 0.00$ ). Therefore, data from both correct corners were pooled for each animal, and the same was done for both incorrect corners (errors). The percentage of geometrically correct choices ( $M = 69.00\%$ , confidence interval [CI]  $\pm 18.41\%$ ) was significantly higher than that expected by chance (Friedman,  $\chi^2(1, N = 11) = 11.00, p < .001, \eta_p^2 = 0.24$ ). Clearly, the toads were able to learn the geometric properties of the goal location even when slope information was present during training. No differences were found between animals that were trained with the reward uphill compared with animals trained with a downhill reward ( $p > .05$ ).

Figure 2C (left) shows the toads' performance on Slope test trials when the geometric information was removed (square arena). With only slope information available, the percentage of choices to the correct corner ( $M = 60.18\%$ , CI  $\pm 17.20\%$ ) was significantly higher than expected by chance (Friedman,  $\chi^2(1, N = 11) = 7.36, p < .007, \eta_p^2 = 0.50$ ). The percentage of errors when animals chose the correct slope direction (uphill or downhill) but the wrong side (e.g., right instead of left;  $M = 18.00\%$ , CI  $\pm 13.41\%$ ) was not different from other types of errors ( $p > .05$ ). Also, no differences were found between animals trained uphill and animals trained downhill ( $p > .05$ ). Paralleling the Geometry-only results, this analysis indicates that the toads were able to display memory for the goal location relying solely on slope even when geometric information was available during training.

The toads' performance on Dissociation/Conflict Test trials, in which the geometric and slope information were set in conflict, is shown in Figure 2D (left). As no difference was found in the percent of choices to the two geometrically correct corners ( $p > .8, F = 0.06$ ), those data were pooled. The choice distribution clearly revealed a preference for the two geometrically correct corners, with a total of 75% of the choices made to them ( $M = 75.00\%$ , CI  $\pm 15.33\%$ ; chance was 50%). Indeed, the percent of choices to the geometrically correct corners were generally in line with the percent of geometric choices on the Geometry test trials (see Figure 2B, left). Choices to geometry were significantly higher than expected by chance (Friedman,  $\chi^2(1, N = 11) = 4.46, p < .035, \eta_p^2 = 0.64$ ). By contrast, only 15% ( $M = 15.00\%$ , CI  $\pm 13.41\%$ ; chance 25%) of the choices were made to the correct location with respect to the learned slope information; the choices to slope did not differ from chance ( $p > .05$ ). Only 9% of the choices were made to the location that could not be a correct either by geometry or slope ( $M = 9.00\%$ , CI  $\pm 12.61\%$ ), which did differ from chance (Friedman,  $\chi^2(1, N = 11) = 4.46, p < .035$ ). Therefore, under conflict conditions, the toads of the current study displayed a robust preference for using geometric information instead of slope information to locate the goal. No differences in choice distribution were found between animals trained uphill and animals trained downhill ( $p > .05$ ).

## Discussion

The results of the experiment show that toads are capable of using both the boundary geometry and slope of an arena to locate

a goal. In a previous study (Sotelo et al., 2015), we showed that toads can independently rely on geometry and a polarizing, visual cue to find a goal. As such, the current study adds slope as a potential source of spatial information toads can rely on for goal localization.

A previous study in amphibians, using a rudimentary horizontal/vertical source of information provided by a four-level caved landscape, was reported by Lüddecke (2003). That study showed that dendrobatid frogs, *Colostethus palmatus*, successfully associated several qualities of the caves in a device with a 24-cave communal paludarium. A gradually decreasing cave-search time to find an available place across trials was observed, suggesting that frogs learn to locate a goal by memorizing their own successfully used motor-patterns in combination with referencing available environmental cues. However, in this study no appropriate controls were conducted, and therefore, it is difficult to interpret the conclusions in the context of the spatial learning.

The use of slope to reorient toward a goal has been studied in other vertebrate species such as pigeons (Nardi & Bingman, 2009a; Nardi et al., 2010) and humans (Nardi et al., 2011, 2013). In contrast to the toads of the current study, however, pigeons preferentially relied on slope when set in conflict with geometry. For the pigeon experiments, subjects were trained in a trapezoidal arena instead of a rectangular-shaped one as with our toads. The interesting feature of a trapezoid shape is that it allows animals to discriminate among each of the four corners by geometry alone. Therefore, the difference between pigeons and toads with respect to preferring geometry or slope may be even more profound as the toads preferred geometry over feature when geometry predicted the goal with only 50% accuracy, while pigeons preferred slope over geometric information even when geometry predicted the location of the goal with 100% accuracy. It is also worth mentioning that hippocampal lesions did not impair pigeons' use of slope in locating a goal (Nardi & Bingman, 2009b).

In the human study of Nardi et al. (2011), a similar slope experiment was carried out in a square enclosure, and therefore, no geometric information could be used to locate the goal. The experiment demonstrated the use of slope information to locate a goal, and also showed a tendency for men to outperform women even in the presence of other sources of information such as visual cues (see also Nardi et al., 2013).

Slope necessarily adds a third dimension to spatial tasks, and there have been other approaches to studying navigation in three dimensions other than slope. Grobety & Schenk (1992a) were among the first to study three-dimensional (3D) navigation in rats, using a type of cube apparatus, with a specific interest in contrasting performance in the vertical in comparison to the horizontal plane. They found that rats could successfully reorient along both planes, but preferred movement along the horizontal. They also hypothesized that learning progressed as a two-step process with vertical being learned before horizontal coordinates. Jovalekic et al. (2011) tested rats using two different tasks (a "foraging" and "detour" task) in a 3D space and they also reported that animals preferred to move in the horizontal dimension rather than the vertical one, and that the two dimensions seemed to be encoded in parallel. Similarly, hummingbirds appear more sensitive to spatial information from the horizontal compared to the vertical plane (Flores-Abreu, Hurlly, & Healy, 2013).

While studies in rats have shown less sensitivity to the vertical compared with horizontal spatial plane, studies in the blind Mexican cave fish, *Astyanax fasciatus*, showed that these animals are capable of spatial learning both in the horizontal and vertical dimensions whether they were presented together or separately. However, they preferred to guide themselves by the vertical information when under conflict (Holbrook & Burt de Perera, 2009).

To date, all vertebrate species tested have displayed some ability to represent vertical space. However, the data presented here, coupled with a survey of the literature, leads to the hypothesis that whereas rats and toads seem to have an impoverished sense of vertical space, pigeons, but perhaps less so hummingbirds, and fish seem as comfortable locating a goal in the vertical plane as they do in the horizontal. The intuitive explanation for the species group differences is that while rats and toads are 2D "surface movers," fish and birds freely move in 3Ds and seem to have evolved neural adaptations to better represent and use vertical space (see Holbrook & Burt de Perera, 2009). Bats are also well suited to navigate in 3D space (see Geva-Sagiv, Las, Yovel, & Ulanovsky, 2015). Having said that, it should be noted that the slope angle used in the toad study was not as steep as the 20° slope used for pigeons by Nardi and colleagues (Nardi & Bingman, 2009a, 2009b; Nardi et al., 2010). As such, slope might have been less salient in the toad study. This point is made more relevant by findings in humans indicating a diminished salience of slope when set to 5° (Nardi et al., 2013, 2015) compared with the 20° slope used in the pigeon studies. Therefore, before a convincing difference in the relative use of slope between pigeons and toads, and more generally surface versus 3D movers, can be accepted, toads should be tested on the steeper slope used in the pigeon studies.

Finally, it is interesting that the number of sessions needed to reach the learning criterion in the current toad study was smaller than what has been observed for other kinds of spatial tasks toads have been tested on (see Daneri et al., 2011, 2015). Even in other studies carried out in the same rectangular enclosure with (Geometry-Feature) and without (Geometry-Only) a visual feature cue, animals needed between 13 and 16 sessions to reach the learning criterion (for a summary of some of these experimental results, see Sotelo et al., 2015). Thus, although slope did not appear to control the behavior of the toads as much as geometry did, it is noteworthy that the combination of slope and geometry facilitated learning compared with toads trained with geometry alone or geometry together with a polarizing feature cue in the same arena (Sotelo et al., 2015). It would seem that the toad brain is better designed for the integrative processing of geometry and slope information compared with geometry and visual feature cues, suggesting that in nature toads routinely rely on slope as a source of navigational information. The question then becomes how does slope facilitate preferential geometric learning? Relevant here are the experimental designs used in studies carried out in fish (Lee, Vallortigara, Ruga, & Sovrano, 2012; Lee, Ferrari, Vallortigara, & Sovrano, 2015) and bumblebees (Lee & Vallortigara, 2015), which identified a generally more limited use of nongeometrical information when geometry can be used to locate a goal. The same designs could be used to model future toad experiments.

In summary, the current study was designed to test for the ability of toads to use slope as source of spatial information to locate a goal, and investigate the relative importance of slope and geometric information for goal localization. Toads were indeed found to

be able to use slope to locate a goal, but the boundary geometry of the experimental arena was found to be preferentially used by the toads when geometric and slope information were set in conflict.

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Received May 8, 2017

Accepted June 12, 2017 ■