

# Goal orientation by geometric and feature cues: spatial learning in the terrestrial toad *Rhinella arenarum*

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**Abstract** Although of crucial importance in vertebrate evolution, amphibians are rarely considered in studies of comparative cognition. Using water as reward, we studied whether the terrestrial toad, *Rhinella arenarum*, is also capable of encoding geometric and feature information to navigate to a goal location. Experimental toads, partially dehydrated, were trained in either a white rectangular box (Geometry-only, Experiment 1) or in the same box with a removable colored panel (Geometry–Feature, Experiment 2) covering one wall. Four water containers were used, but only one (Geometry–Feature), or two in geometrically equivalent corners (Geometry-only), had water accessible to the trained animals. After learning to successfully locate the water reward, probe trials were carried out by changing the shape of the arena or the location of the feature cue. Probe tests revealed that, under the experimental conditions used, toads can use both geometry and feature to locate a goal location, but geometry is more potent as a navigational cue. The results generally agree with findings from other vertebrates and support the idea that at the

behavioral-level geometric orientation is a conserved feature shared by all vertebrates.

**Keywords** Geometric and feature orientation · Spatial learning · Toads

## Introduction

The mechanisms by which animals can locate a goal location following some kind of displacement necessarily require memory for the spatial properties of an environment to be translated into a goal-directed bearing. Among the many mechanisms available, the demonstration by Cheng (1986) that rats preferentially rely on the boundary geometry of an experimental space to locate a goal location was in many ways a watershed discovery. Numerous studies have since been carried out in a variety of species with the intent of determining how extensive geometric-boundary navigation may be and the relative importance of geometric information compared to landmark or feature information (e.g., colored panels or object landmarks). The emergent picture from such studies is not a simple one, but generally, in addition to rats, fish (e.g., López et al. 1999, 2000a; Sovrano et al. 2003; Vargas et al. 2004a), chicks (e.g., Vallortigara et al. 1990), pigeons (Kelly et al. 1998; Vargas et al. 2004b) and humans (e.g., Hermer and Spelke 1994) all readily employ boundary geometry to locate a goal, but the extent to which geometry is weighed more heavily than feature information is variable and may depend on age (Hermer and Spelke 1994), the size of the environmental enclosure (Learmonth et al. 2002; Sovrano and Vallortigara 2006; Sovrano et al. 2007) and species characteristics (Gray et al. 2005). The importance of geometry is also interesting from a neural coding

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perspective because hippocampal formation lesions can eliminate or interfere with geometric learning (rats, McGregor et al. 2004; pigeons, Bingman et al. 2006; Nardi and Bingman 2007; Vargas et al. 2004b) while keeping feature memory for goal locations intact. Indeed, lesions to a likely hippocampal homologue of teleost fish can disrupt navigation by geometry under some experimental conditions (Vargas et al. 2006, 2011), but not others (Vargas et al. 2011).

Amphibians are an underrepresented study group in vertebrate comparative cognition despite the fact that from an evolutionary perspective they 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 fore-brain/hippocampal formation is less complex than in amniotes (e.g., Roth et al. 2007). But amphibian cognition has not gone completely unstudied and has included experiments carried out in the terrestrial toad, *Rhinella arenarum*, demonstrating considerable learning ability (e.g., Daneri et al. 2011; Muzio et al. 2011). Like other toad species (e.g., Sinsch 1987), *R. arenarum* also carry out seasonal migrations to often remote breeding ponds (Gallardo 1974), thus displaying sufficiently rich spatial representational ability to support such navigation. But are these toads, and by extension other amphibian species, capable of using the boundary geometry of an environmental space to locate a goal location? If yes, when boundary geometry and landmark-feature information are available, are toads more reliant on geometric information and are they able to integrate information from geometry and feature to locate a goal? In the present study, we attempt to address these questions by testing terrestrial toads in an experimental design that has been successfully used with other vertebrate groups. Subjects were tested under two different spatial configurations of cues to determine their capacity to encode and use geometric and feature information to locate a goal. We also analyzed the possible similarities and differences in relation to those described in mammals and birds.

## Experiment 1

In this experiment, we explored whether toads were able to use the geometric information of the environment in a place-finding task in a manner similar to mammals and birds. For this purpose, toads were trained to find a goal placed in two corners of a rectangular environment on the basis of the geometrical information provided by the apparatus (similar to that used by Cheng 1986). In the task, locating the goal required toads to determine the spatial relationships between the geometrical properties of the

arena and the goal (two diagonally opposite corners, one 180° from the other).

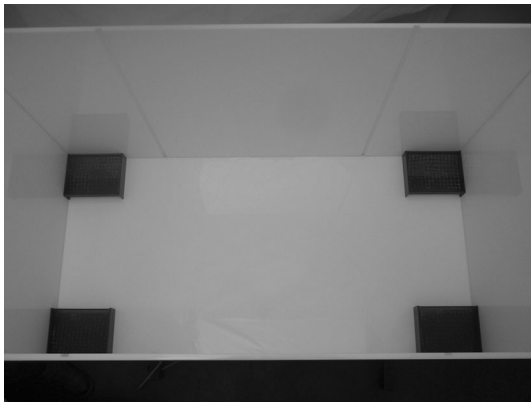
## Method

### Subjects

Ten sexually mature, experimentally naive terrestrial toads (*R. arenarum*), a species not listed as threatened (IUCN 2010), were used. The experimental subjects were captured in ponds around Buenos Aires, Argentina, during September 2012. Animals were maintained according to the guidelines outlined by the NIH Guide for Care and Use of Laboratory Animals. Toads were trained in two groups under the same experimental procedures during November 2012 and January 2013. They were then treated with antibiotics and anthelmintics to avoid bacterial and parasitic infection and kept in group cages with running water during the first month after their arrival in the laboratory. Toads were fed once a week until 1 week before pre-training started. Standard weights (weight of the hydrated animal with its urinary bladder empty; Ruibal 1962) were obtained the day before pre-training. Weights varied between 80.8 g and 139.0 g (mean = 109.81 g, SE ± 6.35 g). No statistical differences in weight were found between the November and January animals; therefore, data from animals from the two groups were pooled. The vivarium was kept at a constant temperature (24–27° C) and humidity (48–52 % RH), and subjected to 16:8 h light/dark cycle (lights on at 06:00 h local time). Toads were trained between 14:00 and 19:00 h. Just prior to pre-training session, animals were transferred to individual enclosures and dehydrated to 80 % of their standard weights. This procedure successfully results in toads motivated to search for water as a primary reinforcer (see for example, Muzio et al. 1992, 2011).

### Training environment

The experimental environment (Fig. 1) consisted of an elevated, rectangular-shaped Plexiglas arena (90 cm long × 45 cm wide × 60 cm high) surrounded by a circular white curtain. For this experiment (Geometry-only), all four walls were white. Four plastic green water containers (13 cm long × 10 cm wide × 3 cm high), which were covered with a wire mesh and filled with deionized water, were placed at each corner of the rectangular arena. Access to the water 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, the 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



**Fig. 1** Rectangular arena for toads trained in Experiment 1 (Geometry-only). Four plastic green water containers were placed at each corner; only two geometrically equivalent (*diagonal*) corners were rewarded with deionized water. There were no visual cues on the walls

surface (inaccessible water). This procedure ensured remote sensory access to the water for both the reinforced and non-reinforced container conditions. During training sessions (but not probe trials, see below), only two of the water containers were filled up to a level accessible to the animals (the geometrically symmetrical goal locations). 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 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 sessions.

#### *Behavioral procedures*

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 it was raised by hand, releasing the toad to move freely in the rectangular arena. Although the animals were not rotated prior to 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 eliminated the use of path integration to locate the goal. Each toad was trained for its three daily 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

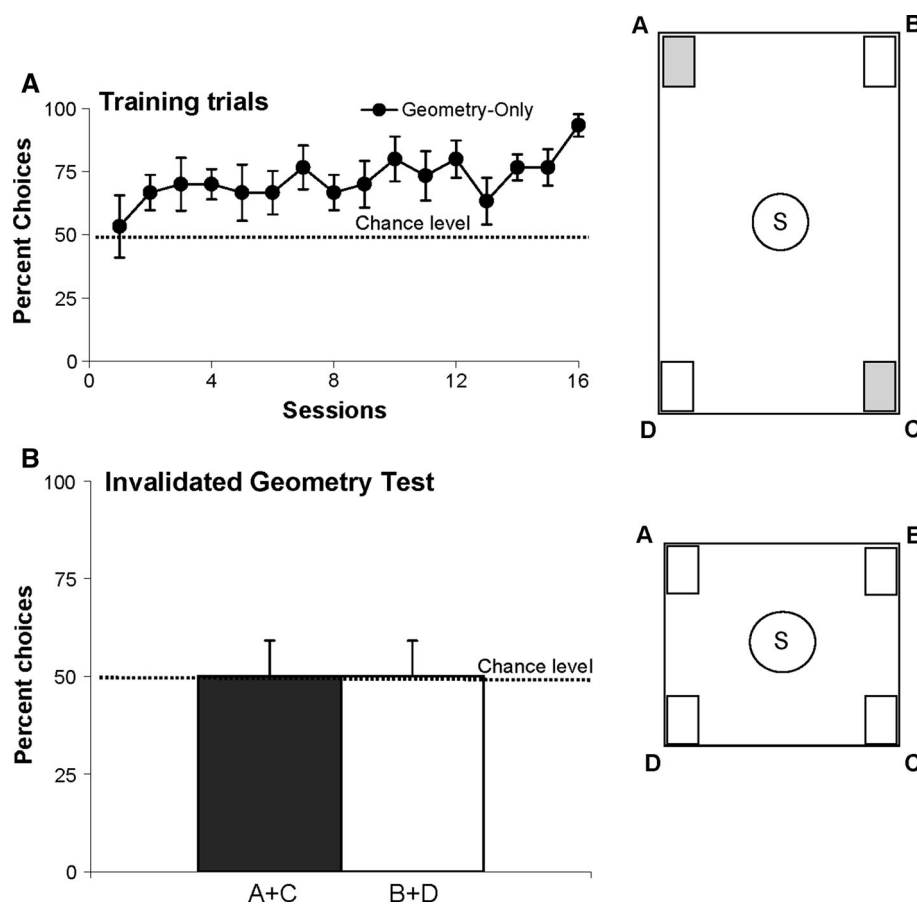
by the animal's standard weight and multiplied by 100 to provide a relative measure of water uptake of each session adjusted by body weight (Daneri et al. 2011; Muzio et al. 1992). After each training session, as a consequence of water uptake during trials, toads increased beyond 80 % of their standard weight. Therefore, before the next session, they were dehydrated again until they reached the target weight.

*Pre-training.* Before beginning training trials, the animals were individually pre-exposed to the rectangular arena twice (one session per day). During pre-training sessions, water was available 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 each group of toads (two groups were used) reached the acquisition criterion (see below). In this experiment (Geometry-only), animals ( $n = 10$ ) were tested as one group of 5 in November, and another group of 5 in January.

Toads were trained in the rectangular arena with four identical white walls. The aim of this training was to determine whether the animals could learn a goal location (the two water-accessible containers) using the boundary geometry of the experimental arena without the aid of a polarizing cue (see Fig. 2a-right). For half the animals (divided between the two groups), the geometrically correct corners had the short wall to the left and long wall to the right (facing the goal-water container), while for the remaining half of the animals the correct corners had the short wall on the right and the long wall on the left. After being released into the arena, toads were free to move for up to 3 min to select a water container. A 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 a 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 nearest correct container (guided correction trial). Each animal was tested for 3 trials per session; inter-trial intervals were spent in the home cage and lasted approximately 2 min. After each trial, the arena was cleaned and pseudorandomly rotated by 90° clockwise or counter-clockwise to ensure that no other cues from the surrounding environment could be used to locate the goal location. The water containers were shifted among the corners as well. As noted above, toads were trained in two groups of 5 animals each, and they were trained until each group of 5 reached the acquisition criterion of 75 % (chance was 50 %) correct choices for three consecutive sessions. 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

**Fig. 2 a** Percentage of correct choices across the sixteen training sessions of Experiment 1. On the right is a diagram of the experimental environment, showing the start container (S) and the location of the two water-reinforcing goals, which are highlighted in light gray (note the position of the diagonally paired goal locations was counter-balanced across the test subjects). **b** Percentage choice distribution to the two diagonal pairs of the square, probe-trial arena (invalidated geometry test). Error bars denote standard errors of the means



intent with 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).

**Probe tests.** Once reaching criterion, animals were moved on to probe trials. The toads were subjected to probe trials to determine if indeed learning was based on the boundary geometry of the arena. Only one probe-trial type was carried out, Invalidated Geometry Test, in which the rectangular arena was turned into a square enclosure by the insertion of two additional white Plexiglas panels (modifying the geometric properties of the experimental arena used during training; see schematic in Fig. 2b-right). The point of the “invalidated geometry test” was to make as certain as possible that uncontrolled cues in the arena environment were not controlling the behavior of the animals. 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, and no water-access reward was given (i.e., all containers contained water inaccessible to the toads). Probe trials were repeated four times (over four sessions) for each individual.

## Results

### Training trials

The mean percentage of correct choices during training for the two goal containers are shown in Fig. 2a-left. Across training, toads increased choice accuracy, reaching criterion on session 16. Performance for sessions 14–16 was all above 75 % correct, which was greater than expected by chance [Friedman,  $\chi^2(3, N = 10) = 21.70, p < .001$ ]. The training trial performance remained steady during the post-criterion sessions.

### Probe tests

Figure 2b-left shows the choice performance of the toads during the probe trials in the square arena (Invalidated Geometry Test) and therefore in the absence of any geometric information to discriminate the four corners. During these trials, no significant differences were observed in the percentage of choices to the pairs of diagonal corners (mean = 50.0 %, SE  $\pm 9.1$  %),  $\chi^2(1, N = 10) = 1 \times 10^{-7}, p < 1$ . The fact that choices were random under this test condition demonstrates that in the absence of geometric information the animals chose randomly among the

four water pools. Clearly, the absence of a discriminative geometry eliminated behavioral discrimination on the part of the toads.

## Experiment 2

A broad range of vertebrate species have been shown to be able to use the boundary geometry of an environment in support of spatial cognition (see Introduction), and based on the results of Experiment 1 above, the terrestrial toad, *R. arenarum*, can now be added to that list. As noted in the Introduction, a considerable amount of research effort has been expended in attempts to understand the relationship between geometry-based and landmark/feature-based goal location behavior in a variety of vertebrate species. Although the available data defy any attempt to develop a globally applicable principle (e.g., geometry always supersedes feature cues in guiding goal location behavior), from a comparative perspective it is still of interest to determine how toads manage the simultaneous availability of geometric and feature cues. With that objective, we performed the following experiment to analyze the orientation strategies of toads when the experimental environment simultaneously provided geometric and feature information. Our intent was to determine which of the two sources of spatial information may have preferential access in controlling the goal location behavior of the toads.

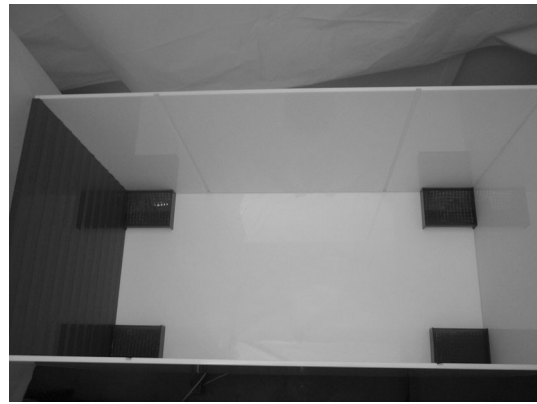
### Method

#### Subjects

Ten sexually mature, experimentally naive terrestrial toads (*R. arenarum*) were used. As in Experiment 1, they were trained in two groups under the same general experimental procedures during November 2012 and January 2013. The anti-parasitic treatment and maintenance of the animals in the laboratory were also the same as in the previous experiment. Weights varied between 74.7 and 163.8 g (mean = 113.25 g, SE  $\pm$  11.00 g). No statistical differences were found in the weights of the animals in the November and January groups; therefore, data from the two groups of animals were pooled.

#### Training environment

The experimental environment (Fig. 3) consisted of the same elevated, rectangular-shaped Plexiglas arena used in the Experiment 1. For the present experiment (Geometry–Feature), however, one of the walls consisted of a panel with alternating blue and red stripes (not controlled for brightness). For Experiment 2, training sessions (but not



**Fig. 3** Rectangular arena for toads trained in Experiment 2 (Geometry–Feature). Four plastic green water containers were placed at each corner (only one rewarded with deionized water). One short wall had a removable colored panel (*red and blue horizontal stripes*) during the training trials (color figure online)

probe trials, see below) were associated with only *one* of the four water containers filled up to a level accessible to the animals (goal location). All other conditions were identical to Experiment 1.

#### Behavioral procedures

General procedures were the same as in Experiment 1.

*Pre-training.* Animals received two pre-training sessions identical to those given in Experiment 1, where the toads were individually pre-exposed to the rectangular arena with all four water containers rewarded. The striped panel was not present during pre-training.

*Training.* Training began the following day (one daily session of three trials) and continued until a group of animals reached the acquisition criterion (see below). In this experiment (Geometry–Feature), animals ( $n = 10$ ) had one group of 5 tested in November and one group of 5 tested in January. Two animals were eliminated from the analysis (one died prior to completion of pre-training and the other because its behavior was highly and unusually variable across the training sessions). Therefore, the final number of the Experiment 2 animals was 8.

Toads were trained using the striped panel inserted over one of the short walls of the rectangular-shaped arena, and only one water container provided access to water. The ultimate goal of the training was to determine whether geometry or feature information was preferentially used by the toads to locate the goal-water container given that geometry could only be used to identify the *two candidate* correct containers and feature could be used to *uniquely* locate the one container that allowed access to water (Fig. 4A-right). But first we needed to determine whether the combination of feature and geometry information

would enable the toads to locate the one goal location. To control for any cues other than the boundary geometry and the striped panel, from trial to trial the panel was shifted between the two short walls and the rectangular arena was rotated pseudorandomly clockwise or counter-clockwise by 90°. As indicated above, toads were trained in two groups of 5 animals each, and training continued until each group of toads reached the acquisition criterion of 50 % correct responses (the presence of the feature cue results in chance being 25 %) for three consecutive sessions. The goal location was balanced among the four corners of the arena. As such, 5 animals were trained with the goal toward the striped panel and 3 animals were trained with the goal away from the striped panel. Goal location was also balanced with respect to being located to the left or the right when facing the target wall. All other procedures were identical to Experiment 1 (Geometry-only) training (see above).

*Probe tests.* After reaching criterion, toads were subjected to three different probe-trial types: (1) Geometry-only Test; (2) Feature-only Test; and (3) Dissociation/Conflict Test (see schematics in Fig. 4b–d, respectively). Each animal was tested three times for each probe-trial type, and on probe trials access to water reward was denied. 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, and no water-access reward was given (in the same way as in Experiment 1). 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-only Test (Fig. 4b-right), the striped panel was removed, rendering all the arena walls white. 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 Feature-only Test (Fig. 4c-right), the rectangular arena was adapted to a square-shaped arena (45 cm long × 45 cm wide × 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 training apparatus, inside the rectangular arena. Across trials, the striped panel was pseudorandomly attached to one of the added panels (two possible locations for each trial). By rendering geometric information irrelevant, the purpose of this test was to determine whether the toads learned to locate the goal location by feature information alone.

For the Dissociation/Conflict Test (Fig. 4d-right), the striped panel used during training sessions was replaced by a larger one (maintaining the same colors and width of stripes), which was rotated 90° in the enclosure to occupy a

long wall. This change resulted in one corner being correct with respect to the feature cue (e.g., the goal is located at the right corner of the striped panel), two corners being correct with respect to geometry and one corner that could not be the goal corner based on either feature or geometry. The purpose of the Dissociation/Conflict Test was to determine whether feature or geometry was more potent in controlling the behavior of the animals when the sources of information were put in conflict (i.e., they separately provided contradictory information with respect to goal location).

## Results

### *Training trials*

Figure 4a-left shows the mean percentage of correct choices to the goal location during the training trials. Across training sessions, toads increased choice accuracy, reaching criterion on session 13 after matching or exceeding 50 % correct choices from sessions 11–13; performance that was higher than that expected by chance [Friedman,  $\chi^2(3, N = 8) = 15.38, p < .01$ ]. Performance levels remained steady during the post-criterion sessions.

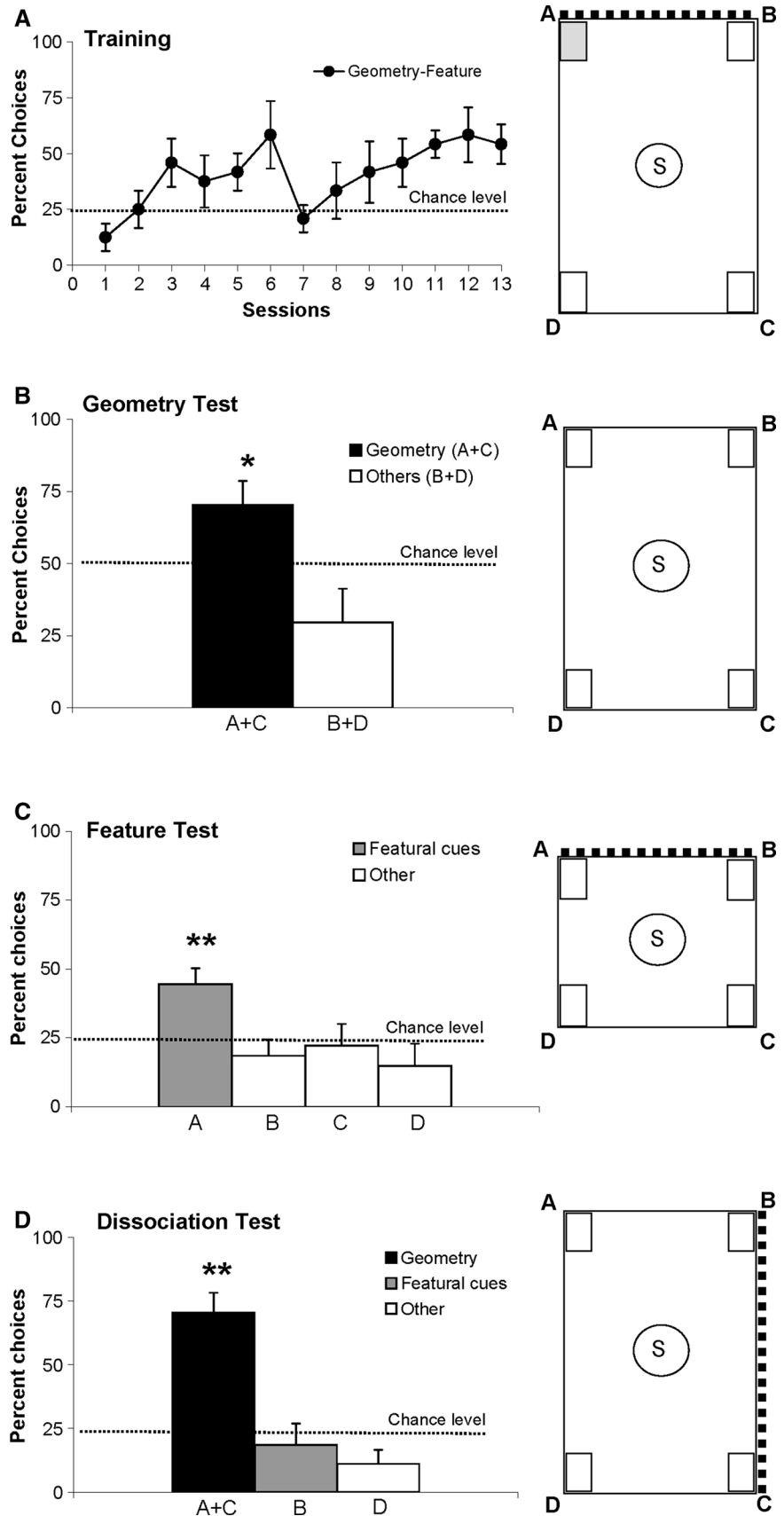
Of potential relevance is whether the toads trained to a goal which had them move toward the feature panel differed from the toads trained to a goal which had them move away from the feature panel. Unfortunately, the animals were generally not coded with respect to whether they were trained toward or away from the feature panel, nor were errors coded with respect to type (e.g., rotational errors). In the few animals where we know the direction of training with respect to the feature panel, there is a modest tendency for those animals trained toward the feature panel to make more correct choices, but the difference is too small to be informative. Given the lack of any substantial difference between the two training conditions, the data from the two groups in this experiment were pooled for all analyses.

### *Probe tests*

Figure 4b-left shows the toads' performance during the Geometry-only Test, when the feature information was removed. The percentage of geometrically correct choices (mean = 79.2 %, SE ± 8.8 %) were significantly higher than that expected by chance,  $\chi^2(1, N = 8) = 4.50, p < .05$ . Clearly, the toads were able to learn the geometric properties of the goal location even when a salient visual landmark could also be used during training.

Figure 4c-left shows the toads' performance during the Feature-only Test, when the geometric information was removed. The percentage of choices to the correct container (mean = 45.8 %, SE ± 6.1 %) were significantly

**Fig. 4** **a** Percentage of correct choices during the thirteen training sessions of Experiment 2. On the right is a diagram of the experimental conditions, showing the start container (S), the position of the colored panel and the location of the goal (in *light gray*). Schematic is shown with the reinforced water container on the left face of the colored panel. However, the actual reinforced water container positions were counterbalanced across toads. **b** Percentage choice distribution and schematic representation of the Geometry-only Test. **c** Percentage choice distribution and schematic representation of the Feature-only test. **d** Percentage choice distribution and schematic representation of the Conflict/Dissociation test. *Error bars* denote standard errors of the means. *Asterisks* denote significant differences between choices and chance ( $*p < .05$ ;  $**p < .01$ )



higher than expected by chance when only the feature information was available,  $\chi^2(1, N = 8) = 8.00, p < .01$ . Similar to the Geometry-only probe, the data indicate that the toads were able to learn the location of the goal based on the feature panel even when geometric information was also available during training.

The toads' performance during the potentially more revealing Dissociation/Conflict Test, in which the geometric and feature information were set in conflict, is shown in Fig. 4d-left. The choice distribution clearly revealed a preference for the two geometrically correct corners, with 75.0 % (SE  $\pm$  8.3 %) of the choices made to them. Indeed, the percent of choices to geometry were generally in line with the percent geometric choices on the Geometry-only probe trials. Choices to geometry were significantly higher than expected by chance,  $\chi^2(1, N = 8) = 8.00, p < .01$ . By contrast, only 16.7 % (SE  $\pm$  8.9 %) of the choices were made to the correct feature location, a value that did not differ from chance,  $\chi^2(1, N = 8) = 0.50, p < .48$ . Therefore, under conflict conditions, the toads of the current study displayed a robust preference for using geometric information.

## Discussion

The results of Experiment 1 demonstrate that toads were able to locate a goal in an environment that lacked relevant feature information by encoding the goal location in relation to the geometric properties of the experimental arena. At the end of training, the toads were preferentially choosing one of the two geometrically equivalent corners associated with the water reward (Fig. 2a). The results suggest that the toads relied on the geometric information provided by the surfaces of the experimental apparatus to orient toward the goal locations. Thus, when the geometric characteristics of the apparatus were rendered ambiguous (square), choice performance fell to chance, indicating that the toads could not use any uncontrolled, non-geometric information to identify the goal locations (Fig. 2b).

The results of Experiment 2 confirm the conclusion of Experiment 1 in demonstrating that toads can rely on the boundary geometry of an environment to locate a goal. Toads trained with both geometric and feature information were also able to simultaneously use both types of spatial information for goal location. The performance during training trials shows that the toads used, at least partially, the feature information to locate the goal because they consistently preferred the one, correct goal location, compared to its geometric equivalent, and on Feature-only probe trials, without geometric information, they preferentially chose the correct location with respect to the environment's feature panel [ $\chi^2(1, N = 8) = 6.00,$

$p < .05$ ; Fig. 4c-left]. The fact that toads encoded both geometric and feature information conveyed by the environment indicates that they, like most vertebrate species studied, can use a variety of diverse and seemingly redundant sources of spatial information to locate a goal. Of particular interest was the Dissociation/Conflict Test, when the feature panel was rotated 90° relative to the geometric cues within the experimental arena (Fig. 4d-right) and the two sources of spatial information provided contradictory information about the location of the goal. Here, the toads preferentially chose on the basis of the geometric information [Friedman,  $\chi^2(1, N = 8) = 4.5, p < .05$ ], suggesting that they had encoded the geometric and feature information as two independent, competing strategies rather than integrating them into one coherent goal representation.

In summary, the most important findings of the current study are that terrestrial toads can rely on the boundary geometry of an environment to locate a goal location, and when trained with an alternative and more predictive source of spatial information, a feature cue, they preferentially rely on geometry.

As we note in the Introduction, the use of boundary geometry for goal localization across different species and experimental conditions is not straightforward. It is for this reason that we emphasize that the conclusions we draw should not be generalized to other experimental contexts without some empirical justification. Indeed, numerous theories have been developed to explain *how* geometry is used to locate a goal (e.g., Miller and Shettleworth 2008; Pecchia and Vallortigara 2010; Sovrano and Vallortigara 2006; see review by Cheng et al. 2013). However, because our study was designed to offer a first test of geometric navigation in toads without consideration of exactly how geometry would be used, our data by themselves are not suited to discriminate among the different theories.

From a comparative perspective, the relationship between geometry and feature information and their neural encoding is of particular interest (see Introduction). For example, some fish species can solve a directly cued task by means of a guidance strategy, with their performance reliant on information closely associated with the goal and independent of the geometry of the apparatus. In such a task, no positive learning transfer is observed during reversal. Moreover, data from fish with brain lesions reveal that telencephalic, presumptive hippocampal ablation produces selective impairments in a spatial constancy task, whereas it has no significant effect on a directly cued task (López et al. 2000b; Salas et al. 1996). Interesting in this context is that hippocampal formation lesions also eliminate or interfere with geometric learning in a variety of vertebrate species (rats, McGregor et al. 2004; pigeons, Bingman et al. 2006; Nardi and Bingman 2007; Vargas



et al. 2004b) while keeping feature memory for goal locations intact. Indeed, lesions to the presumptive hippocampal homologue of teleost fish can also disrupt navigation by geometry (Vargas et al. 2006; but see Vargas et al. 2011). As such, one would expect that the toad medial pallium, the homologue of the mammalian hippocampus, would also be important for the geometric learning reported in the current study. Consistent with this expectation, toads with lesions of the medial pallium were impaired in using visual guidance but not an egocentric turn response in choosing the correct arm of a T-maze (Daneri Casanave and Muzio in preparation).

In conclusion, the present work reveals that toads, similar to birds, mammals and fish, can encode the geometric properties of an environment and use that information to locate a goal. In fact, toads can use both geometric and feature information for navigation. Furthermore, when both geometric and feature information are presented together, toads seem to encode cooperatively but separately both types of information. However, in the current experimental setting, when a conflict is created between the now two competing spatial representations, geometry is preferred over feature.

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## References

- Bingman VP, Erichsen JT, Anderson JD, Good MA, Pearce JM (2006) Spared feature-structure discrimination but diminished salience of environmental geometry in hippocampal lesioned homing pigeons (*Columba livia*). *Behav Neurosci* 120(4):835–841
- Cheng K (1986) A purely geometric module in the rat's spatial representation. *Cognition* 23:149–178
- Cheng K, Huttenlocher J, Newcombe N (2013) 25 years of research on the use of geometry in spatial reorientation: a current theoretical perspective. *Psychon Bull Rev* 20:1033–1054
- Daneri MF, Casanave E, Muzio RN (2011) Control of spatial orientation in terrestrial toads (*Rhinella arenarum*). *J Comp Psychol*. doi:10.1037/a0024242
- Gallardo JM (1974) *Anfibios de los alrededores de Buenos*. Ed. Eudeba, Buenos Aires
- Gray ER, Bloomfield LL, Ferrey A, Spetch ML, Sturdy CB (2005) Spatial encoding in mountain chickadees: features overshadow geometry. *Biol Lett* 1:314–317
- Hermer L, Spelke ES (1994) A geometric process for spatial reorientation in young children. *Nature* 370:57–59
- Kelly DM, Spetch ML, Heth CD (1998) Pigeons' (*Columba livia*) encoding of geometric and featural properties of a spatial environment. *J Comp Psychol* 112:259–269
- Learmonth AE, Nadel L, Newcombe NS (2002) Children's use of landmarks: implications for modularity theory. *Psychol Sci* 13:337–341
- López JC, Broglio C, Rodríguez F, Thinus-Blanc C, Salas C (1999) Different learning strategies in goldfish trained in a spatial constancy task versus a cued one. *Anim Cogn* 2:109–120
- López JC, Bingman VP, Rodríguez F, Gómez Y, Salas C (2000a) Dissociation of place and cue learning by telencephalic ablation in goldfish. *Behav Neurosci* 114:687–699
- López JC, Broglio C, Rodríguez F, Thinus-Blanc C, Salas C (2000b) Reversal learning deficit in a spatial task but not in a cued one after telencephalic ablation in goldfish. *Behav Brain Res* 109:91–98
- McGregor A, Hayward AJ, Pearce JM, Good MA (2004) Hippocampal lesions disrupt navigation based on the shape of the environment. *Behav Neurosci* 118(5):1011–1021
- Miller NY, Shettleworth SJ (2008) An associative model of geometry learning: a modified choice rule. *J Exp Psychol Anim B* 34:419–422. doi:10.1037/0097-7403.34.3.419
- Muzio RN, Segura ET, Papini MR (1992) Effect of schedule and magnitude of reinforcement on instrumental acquisition and extinction in the toad, *Bufo arenarum*. *Learn Motiv* 23:406–429
- Muzio RN, Pistone Creydt V, Iurman M, Rinaldi MA, Sirani B, Papini MR (2011) Incentive or habit learning in amphibians? *PLoS ONE* 6(11):e25798. doi:10.1371/journal.pone.0025798
- Nardi D, Bingman VP (2007) Asymmetrical participation of the left and right hippocampus for representing environmental geometry in homing pigeons. *Behav Brain Res* 178:160–171
- Pecchia T, Vallortigara G (2010) View-based strategy for reorientation by geometry. *J Exp Biol* 213:2987–2996. doi:10.1242/jeb.043315
- Roth G, Laberge F, Mühlenbrock-Lenter S, Grunwald W (2007) Organization of the pallium in the fire-bellied toad *Bombina orientalis*. I: morphology and axonal projection pattern of neurons revealed by intracellular biocytin labeling. *J Comp Neurol* 501:443–464
- Ruibal R (1962) The adaptive value of bladder water in the toad (*Bufo cognatus*). *Physiol Zool* 35:218–223
- Salas C, Broglio C, Rodríguez F, López JC, Portavella M, Torres B (1996) Telencephalic ablation in goldfish impairs performance in a 'spatial constancy' problem but not in a cued one. *Behav Brain Res* 79:193–200
- Sinsch U (1987) Orientation behaviour of toads (*Bufo bufo*) displaced from the breeding site. *J Comp Physiol A* 161:715–727
- Sovrano VA, Vallortigara G (2006) Dissecting the geometric module. A sense linkage for metric and landmark information in animals' spatial reorientation. *Psychol Sci* 17:616–621
- Sovrano VA, Bisazza A, Vallortigara G (2003) Modularity as a fish views it: conjoining geometric and nongeometric information for spatial reorientation. *J Exp Psychol Anim B* 29:199–210
- Sovrano VA, Bisazza A, Vallortigara G (2007) How fish do geometry in large and in small spaces. *Anim Cogn* 10:47–54
- Vallortigara G, Zanforlin M, Pasti G (1990) Geometric modules in animals' spatial representation: a test with chicks. *J Comp Psychol* 104:248–254
- Vargas JP, López JC, Salas C, Thinus-Blanc C (2004a) Encoding of geometric and featural spatial information by Goldfish (*Carassius auratus*). *J Comp Psychol* 118:206–216
- Vargas JP, Petruso E, Bingman V (2004b) Hippocampal formation is required for geometric navigation in pigeons. *Eur J Neurosci* 20:1937–1944
- Vargas JP, Bingman VP, Portavella M, López JC (2006) Telencephalon and geometric space in Goldfish. *Eur J Neurosci* 24:2870–2878
- Vargas JP, Portavella M, Quintero E, López JC (2011) Neural basis of the spatial navigation based on geometric cues. *Behav Brain Res* 225:367–372. doi:10.1016/j.bbr.2011.07.027