

Use of Local Visual Cues for Spatial Orientation in Terrestrial Toads (*Rhinella arenarum*): The Role of Distance to a Goal

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The use of environmental visual cues for navigation is an ability present in many groups of animals. The effect of spatial proximity between a visual cue and a goal on reorientation in an environment has been studied in several vertebrate groups, but never previously in amphibians. In this study, we tested the use of local visual cues (beacons) to orient in an open field in the terrestrial toad (*Rhinella arenarum*). Experiment 1 showed that toads could orient in space using 2 cues located near the rewarded container. Experiment 2 used only 1 cue placed at different distances to the goal and revealed that learning speed was affected by the proximity to the goal (the closer the cue was to the goal, the faster toads learned its location). Experiment 3 showed that the position of a cue results in a different predictive value. Toads preferred cues located closer to the goal more than those located farther away as a reference for orientation. Present results revealed, for the first time, that (a) toads can learn to orient in an open space using visual cues, and that (b) the effect of spatial proximity between a cue and a goal, a learning phenomenon previously observed in other groups of animals such as mammals, birds, fish, and invertebrates, also affects orientation in amphibians. Thus, our results suggest that toads are able to employ spatial strategies that closely parallel those described in other vertebrate groups, supporting an early evolutionary origin for these spatial orientation skills.

Keywords: spatial learning, local visual cues, beacons, toads

Staying in one place or moving to another may mean accessing a resource or avoiding a predator. In this sense, choosing where to settle and how to move to a new location is clearly relevant for an

animal. Much evidence suggests that natural selection has shaped these decisions adaptively, and certain mechanisms are required to ensure that they are effective. With the goal of reaching a desirable location, an animal needs to decide where to go and how to get there (Barnard, 2004).

Animals use a range of strategies to navigate to a specific goal. These include the use of a single salient cue (a beacon, usually a large local cue near the goal), path integration, learning a sequence of responses, and creating a global, spatial representation of environmental cues (a cognitive map). In the latter case, the goal is defined by its spatial relationship to a number of different landmarks (usually distal cues that surround an experimental space); such a map is highly adaptive, as the removal of any single landmark does not necessarily disrupt navigation (Shettleworth, 2010). Another possibility might be the use of a view-matching strategy of reorientation, in which animals would navigate so as to minimize the difference between the panoramic image of the rewarded site and the panorama perceived from the current location. Recent comparative research on insects and computer modeling suggest that this strategy could be used by both insects and vertebrates (Pecchia & Vallortigara, 2010). According to Tommasi, Chiandetti, Pecchia, Sovrano, and Vallortigara (2012), when encoding distance and direction from simple objects, the simplest form of spatial orientation is that involving a goal directly associated with a visual object. When the spatial location of a landmark coincides with the goal, the orientation behavior simply requires

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that the navigating organism recognizes the landmark in its local space and moves toward it.

There are studies conducted in vertebrates that focus on the use of proximal visual cues to guide navigation. For example, Ingle and Sahagian (1973) revealed that goldfish can learn to swim in a constant direction relative to proximal visual cues within a small enclosure; this response was observed even if goldfish approached the choice point from different directions (Salas, Rodríguez, Vargas, Durán, & Torres, 1996). Fish also revealed sensitivity to environmental shape and dimensions in spatial navigation (such as encoding of geometry and size of the experimental space; see, e.g., Lee, Vallortigara, Flore, Spelke, & Sovrano, 2013; Sovrano, Bisazza, & Vallortigara, 2005, 2007). López and colleagues (2000, 2001) revealed that semiaquatic turtles in a cue guidance procedure solved a task by directly approaching a single, individual intramaze cue associated with a goal. If we analyze the phenomena, it seems that the behavioral control by a visual cue increases as the absolute spatial proximity between the cue and a goal decreases. There is evidence that animals rely preferentially on closer cues to locate a goal (e.g., the work in digger wasps by Tinbergen & Kruyt, 1938). Spatial contiguity favors cues at the goal or very near the goal as the best predictors of its location in rats (Chamizo & Rodrigo, 2004). The fact that animals with the cue positioned closer to the goal learned the task faster has also been observed in other mammals such as dogs (Milgram et al., 1999). This phenomenon seems to be quite general because it is also present in invertebrates such as honeybees (Cheng, Collett, Pickhard, & Wehner, 1987). Furthermore, Spetch (1995) showed in pigeons and humans that the control over the response acquired by a cue at a given distance from the target could be reduced or overshadowed by the presence of another cue closer to the target. The same result was also observed in European jays, a food-storing corvid (Bennett, 1993). Chamizo, Manteiga, Rodrigo, and Mackintosh (2006) studied the competition between cues in spatial learning by rats, analyzing the role of distance to the goal. The spatial proximity of a cue to the platform (the goal) affected not only how well it could be used to locate the platform (better when it was near), but also its ability to prevent learning about other cues (worse when another cue was introduced to form a new configuration). Similar results were also observed in invertebrates. In the study by Cheng et al. (1987), honeybees, trained to forage at a small source of sucrose placed at a constant location within a particular array of cues, weighed nearer cues more heavily than distant ones.

But knowledge about spatial learning in amphibians so far is scarce. This fact is surprising because this is a particularly important group from an evolutionary standpoint given that it lies at the transition between aquatic and terrestrial vertebrates (Macphail, 1982; Muzio, 1999, 2013). Nevertheless, some work has been published demonstrating amphibian spatial learning ability. For example, Adler (1980) showed that the green frog (*Rana calamitans*) is capable of using static visual cues and memorized motor patterns as orientation cues when trained and tested repeatedly in an arena. Lüddecke (2003) also reported spatial learning in the dendrobatid frog (*Colostethus palmatus*), using a situation in which frogs had to orient within an arena with 24 different locations. In another study, Sinsch (1987), using a displacement test in the common toad (*Bufo bufo*), established that vision (use of environmental visual cues) was necessary to orient back to the start

location. Another work by Dall'antonia and Sinsch (2001), in the natterjack toad (*Bufo calamita*), revealed that this species' orientation abilities relied on multisensory cues (visual, magnetic, olfactory) in an octagonal arena. Finally, previous series of experiments on spatial learning performed at our lab (Daneri, Casanave, & Muzio, 2011) revealed that the terrestrial toad (*Rhinella arenarum*) may use a fixed visual cue to solve a spatial challenge on a plus maze. In this case, experimental toads learned to find a container full of water directly cued by a proximal, intramaze visual cue (beacon) located at the end of a reinforced maze arm. With the exception of these earlier studies, there is no other information about maze learning ability using visual cues in amphibians.

To determine the ability of amphibians to use local visual cues, and to reveal whether there is any effect of cue proximity to the goal, we conducted three experiments in an open field setting. Experiment 1 studied how toads orient in an arena using visual cues located near a reward, Experiment 2 revealed the effect of different distances between a cue and the goal, and Experiment 3 studied the competition between two cues in relation to their distance to a goal.

Experiment 1: Use of Local Cues

The objective of our first experiment was to test the spatial abilities of the toad in a circular arena (an open field) and the use of proximal visual cues (beacons) for spatial orientation.

Method

Subjects. The subjects were 11 experimentally naive adult male toads (*Rhinella arenarum*; formerly *Bufo arenarum*), captured in ponds around Bahia Blanca, Argentina. This species is not listed as threatened (International Union for Conservation of Nature and Natural Resources, 2014). Toads were maintained according to the guidelines outlined by the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 2011). Upon their arrival at the laboratory, toads were placed in cages (30 cm long, 21 cm wide, and 21 cm high), five to 10 toads per cage, where they remained with continually running tap water for at least 2 weeks. During the first week, toads were treated with antibiotics and antiparasitics mixed with commercial frog food (approximately 3 g per day per toad). Then, toads were fed only with frog food (once a day during the second week, and after that, once a week). The vivarium was kept at a temperature between 21°C and 23°C, and under a 16:8-hr light–dark cycle (light from 0500 to 2100). Before the start of the experiment, toads were transferred to individual cages with ad libitum deionized water. The day before pretraining, standard weights (weight of the hydrated toad with its urinary bladder empty; Ruibal, 1962) of all toads were obtained. These weights varied between 50 and 102 g. To induce water-search behavior, toads were dehydrated to 80% of their standard weights at the beginning of each pretraining and training session (a standard method employed in this species; e.g., see Muzio et al., 2011; Muzio, Segura, & Papini, 1992).

Apparatus. The experimental environment was composed as an open field made of opaque white Plexiglas; the floor was made of light brown rubber. The open field was 86 cm of diameter and its walls were 75 cm high. On the floor, four positions (goal points)

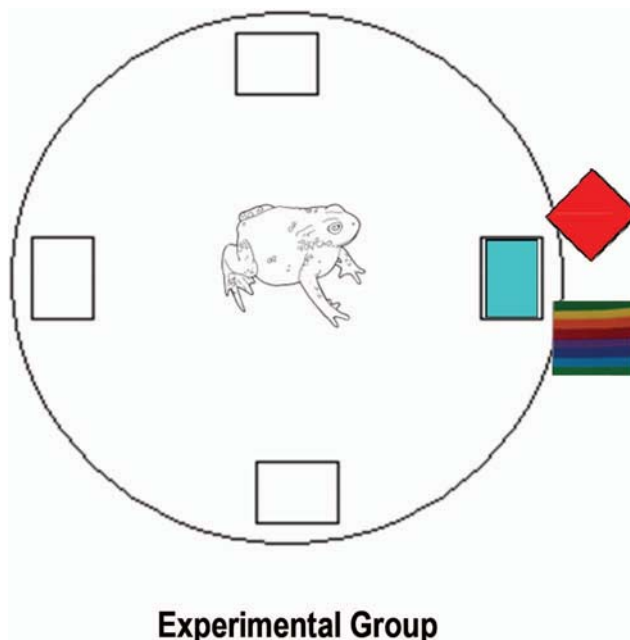
were established (North, West, East, and South). A plastic green water container (13 × 10 × 3 cm, length × width × height) was placed in each of the four goal points against the walls. The water containers were filled with deionized water, but accessibility to the water was controlled by adjusting the water level relative to a metallic grid placed into the container that served as substrate (water level reached the surface of the grid or was underneath). This ensured similar moisture level near both reinforced and nonreinforced containers.

The maze was surrounded by a white curtain that isolated it from any external visual cues. The experimenter was hidden behind the curtain to observe and visually record the toads' behavior (through a small window). Only one observer recorded the data of all the experiments. The arena and the window positions were randomly rotated between experimental sessions. The open field was cleaned between trials to avoid the use of any uncontrolled intramaze cues. Training was carried out in an experimental room kept at a constant temperature and humidity (21–23°C, 48–52% relative humidity) and constant background white noise (20–30,000 Hz).

Procedure. To accustom the toads to obtain water from the plastic green containers in the experimental open field, all the toads received two pretraining sessions of 5 min (one per day). During these sessions, toads were free to move inside the open field and had access to deionized water in all the plastic containers. Training started on the following day. Each toad received 32 training sessions (one session per day of three trials each).

Toads were randomly assigned to one of the following groups: experimental ($n = 6$) and control ($n = 6$). Standard weights did not differ significantly between groups, $F(1, 9) = 0.14, p > .5$. One of the toads in the experimental group died of unknown causes during training (thereby, this group remained with $n = 5$). Toads of the experimental group were trained under a procedure in which the goal (the plastic container full of accessible deionized water) was surrounded by two visual cues: a red rhombus (10 × 10 cm) on the left (when facing the goal container) and a multicolored horizontally striped square (10 × 10 cm) on the right (see Figure 1). Both cues were made of a rubber sheet and placed on the wall 10 cm away and above the edge of the reinforced container. The position of the reinforced container (with the visual cues) was randomly rotated between trials. Containers with unreachable water were placed at the other three goal points. This procedure was designed to prevent the toads from being guided to the correct goal box by a humidity gradient. For toads in the control group, the reinforced container was randomly located at any of the four goal points with no visual cues associated with it. The use of the proximal visual cues for spatial orientation was necessary to successfully solve the task for the experimental group.

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 a start container (12 × 12 × 8 cm, length × width × height) in the center of the open field. It remained under the start container for 30 s until it was raised by hand, releasing the toad to move freely in the open field and to choose among the four containers. As a consequence of their movements during the 30-s waiting period, toads started each trial haphazardly, facing different directions. Furthermore, prior to each trial, toads were placed into the open field from different positions and oriented in different directions in the start container, effec-



Experimental Group

Figure 1. Diagram of the open field environment used, showing the spatial relationship among the four plastic green water containers (placed North, West, East, and South), only one of which (light blue) would be accessible to deionized water as reward. Toad drawing indicates the central start position used at the beginning of each trial. Schematic is shown for the experimental group condition of Experiment 1, with the two visual cues on the walls (a red rhombus on the left and a multicolored horizontally striped square on the right when facing the cues and the reinforced water container). The actual reinforced water container position (with the visual cues) was randomly rotated across trials. For the control group, there were no visual cues on the walls. See the online article for the color version of this figure.

tively eliminating path integration or any other source of information to locate the goal other than the two visual cues placed on the wall. Toads were then allowed to search in the open field until one container was chosen (placing at least one limb on it) or for a maximum of 3 min. The choice was recorded as correct or incorrect based on whether or not the toad arrived at the container with reachable water. If the toad chose the correct container, 2 min of water access were used as reinforcement. In the case of an incorrect choice (a container with unreachable water), the toad was left for 1 min on the metallic grid of the container, and then was gently relocated back to the center of the open field and guided by hand to the correct container to gain access to the water for 2 min (guided trial procedure).

Weight variation (g/100 g) was also recorded. Toads were weighed (in grams) before and after each trial to assess the amount of water uptake that occurred during the trial. The difference between these two weights was divided by the standard weight computed before the first pretraining session and multiplied by 100 to provide a relative measure of water uptake corrected for individual differences in body weight, as done in previous studies (e.g., Muzio et al., 1992).

When the trial was finished, the toad was removed from the maze and returned to its home cage for a 2-min intertrial interval.

This movement also served to disorient the toads between trials. An acquisition criterion of a minimum of five of nine correct choices during three consecutive sessions was used (i.e., 55.56% correct trials in the last three sessions, thus exceeding by more than twice what would be expected by chance). After 24 training sessions, the criterion was reached, and an overtraining and testing phase then began (eight additional sessions). During this period, toads received four trials per session: three training trials plus one probe trial. Presentation of the probe trials was distributed pseudorandomly among the training trials (probe trials never occurred on the first or last trial). Probe trials were designed to rule out that the toads were locating the goal using only visual cues emanating from the correct water container; therefore, during these trials, all the containers were in the unreachable water condition (i.e., during probe trials toads were not reinforced). For each probe trial, the container chosen was recorded and the response classified as correct or incorrect with respect to the original position of the reachable container during training trials.

Analysis of variance (ANOVA) with repeated measures, followed by pairwise comparisons of groups based on the least significant difference test, was applied for statistical analysis of the data. In all cases, significance was evaluated by setting the alpha value at less than .05.

Results and Discussion

Figure 2 presents the percentage of correct responses per training session for each group. Each data point is the average of the data collected from the toads of a particular group. We conducted an ANOVA with a between-subjects factor (condition) and a within-subject factor (session number). An overall analysis of the acquisition period showed a significant effect for group, $F(1, 9) = 66.94, p < .001, \text{partial } \eta^2 = .88$. In the first session, both groups displayed a similar performance, $F(1, 9) = 0.04, p > .5$, and did not choose any of the four containers more than expected by chance (groups vs. chance), $F(2, 14) = 0.17, p > .5$. But during the last three acquisition sessions (22, 23, and 24), the performance of the experimental group was significantly better than that of the

control group, $F(1, 9) = 14.27, p < .01, \text{partial } \eta^2 = .61$. Toads in the experimental group usually began the trial wandering around the start area and then facing toward the container of choice. Performance of this group suggests that they learned how to locate the goal, in contrast with the poor learning of the control group. In addition, during the last three acquisition sessions, toads from the experimental group also showed a clear preference for the correct quadrant associated with the rewarded container directly cued by the two visual cues (58% of choices; see Figure 3A). In contrast, toads of the control group chose equally across all quadrants.

As typically observed (e.g., Muzio et al., 1992), weight variation (measured on a trial-by-trial basis) demonstrated that reward resulted in a significant amount of body weight change that paralleled the toads' better performance across training trials (data not shown). Weight variation means (g/100 g) were 1.98 ± 0.09 for the experimental group and 1.47 ± 0.05 for the control group.

The general performance of both experimental and control groups during the overtraining and testing period was not affected by the introduction of the probe trials (i.e., significant differences between groups were maintained), $F(1, 9) = 32.16, p < .001, \text{partial } \eta^2 = .78$, and no changes across sessions nor interactions were observed ($ps > .5$). These probe trials were applied to determine whether toads solved the task on the basis of a visual cue-guided response. An ANOVA revealed significant differences in the mean correct choices of both groups across test trials, $F(1, 14) = 39.41, p < .001, \text{partial } \eta^2 = .73$ (see Figure 3B). This fact indicates that the visual cues had controlled the toads' behavior in the experimental group, guiding them toward the goal container.

Experiment 2: Effect of Distance From a Visual Cue to the Goal

In the previous experiment, we showed that toads are capable of using visual cues for spatial orientation. Experiment 2 explored the effect of the distance from a visual cue to the goal on learning performance.

Method

Subjects and apparatus. Eighteen experimentally naive adult male toads were obtained and maintained as described in the previous experiment. Standard weights varied between 98 and 125 g and were not statistically different across groups, $F(2, 15) = 0.09, p > .5$. Other conditions of maintenance and apparatus used were the same as those in Experiment 1.

Procedure. Pretraining was the same as that described in Experiment 1. Training started the following day. All toads received a total of 24 training sessions (one session per day, also of three trials each). Toads were randomly assigned to one of three groups: above ($n = 6$), near 10 ($n = 6$), and near 30 ($n = 6$). Toads of the above group had the accessible water container directly cued by a visual cue (beacon) located 10 cm above the container on the wall of the open field. Toads of the near-10 and near-30 groups had the cue displaced 10 and 30 cm, respectively, to the right (when facing the water container; see Figure 4). The cue used in all cases was a multicolored horizontally striped square (10×10 cm) made of rubber (one of the cues used in Experiment 1). For all trials, the cue and associated container were placed at randomly changing goal points relative to the starting location. Similar containers with

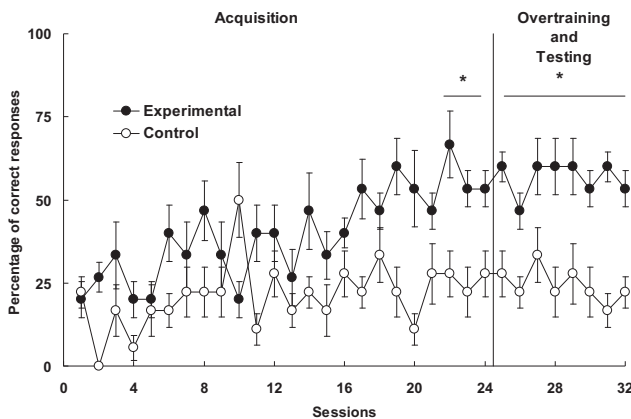


Figure 2. Percentage of correct responses during acquisition (24 sessions) and overtraining and testing (eight sessions) in the experimental ($n = 5$) and control ($n = 6$) groups of Experiment 1. Means and confidence intervals ($\alpha = .05$) are plotted.

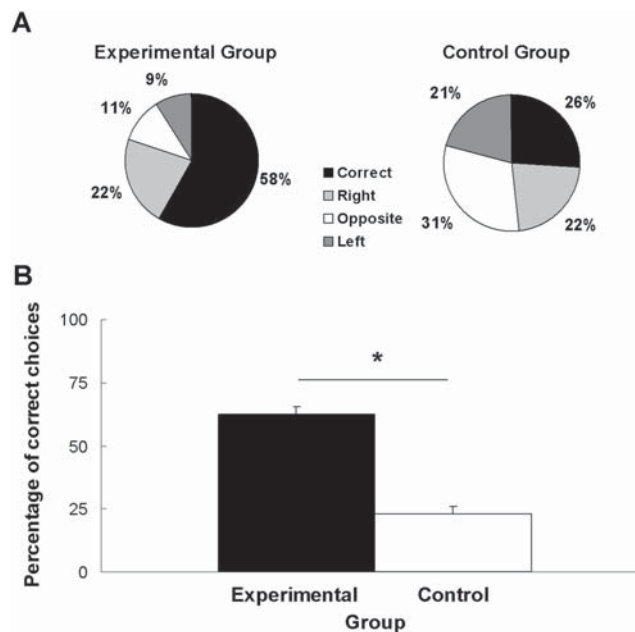


Figure 3. (A) Distribution of percentage of correct quadrant choices across the last three acquisition trials (i.e., when toads had reached criterion) for the experimental and control groups of Experiment 1. Quadrants were differentiated as correct (containing the rewarded container), and right, left, and opposite (according to relative position with respect to the correct container quadrant). (B) Mean percentage of correct choices across test trials (eight tests) for both groups. Means and confidence intervals ($\alpha = .05$) are plotted.

unreachable water were always placed at the other three goal points. Thus, toads could only solve the task using the visual cue as a reference. Toads could not use the presence of reward as cue because, as in Experiment 1, it also ensured the constancy of sensory cues from water in the four containers, both reinforced and nonreinforced (see Apparatus and Procedure sections of Experiment 1, reachable/unreachable water). Other procedural details were the same as described in Experiment 1.

Results and Discussion

Figure 5A shows the percentage of correct responses for each group. An overall analysis of the training period revealed significant effects for group, $F(2, 15) = 22.19, p < .001$, partial $\eta^2 = .74$, across sessions, $F(23, 345) = 6.35, p < .001$, partial $\eta^2 = .30$, but no interaction between factors, $F(46, 345) = 0.85, p > .5$.

Weight variation means (g/100 g) were also recorded and were 1.38 ± 0.08 for the above group, 1.20 ± 0.07 for the near-10 group, and 1.32 ± 0.08 for the near-30 group.

To identify the source of the difference in correct choices observed among the groups, we analyzed the number of sessions necessary to reach the learning criterion (see Figure 5B). An ANOVA revealed significant differences among the groups, $F(2, 15) = 45.90, p < .001$, partial $\eta^2 = .86$; all three groups required a different number of sessions to reach criterion (least significant difference test; all comparisons $p < .005$). These results reveal that the learning rate changed with the distance between the goal and

the visual cue: The closer the visual cue to the goal, the faster the toad learned to locate the goal (i.e., fewer sessions to reach criterion).

Experiment 3: Predictive Value and Competition Between Visual Cues

The fact that toads weighed nearer cues more heavily than distant ones was previously observed in a wide variety of groups of animals (see the introductory section) but never before in amphibians. Thus, Experiment 3 was designed to test in toads the competition between two visual cues in relation to their distance to the goal, analyzing how the distance to the goal affects the predictive value of each cue.

Method

Subjects and apparatus. Fourteen experimentally naive adult male toads were obtained and maintained as described in the previous experiment. Standard weights varied between 80 and 130 g and were not statistically different across groups, $F(1, 8) = 0.30, p > .5$. Other conditions of maintenance and the apparatus used were the same as those in the previous experiments.

Procedure. Pretraining was the same as that described in Experiment 1. Training started the following day. This training protocol consisted of the same training session procedure as in the two previous experiments (one session per day of three trials each). Training was divided into two periods: acquisition (number of sessions to reach the learning criterion of five of nine correct choices during three consecutive sessions) and overtraining and

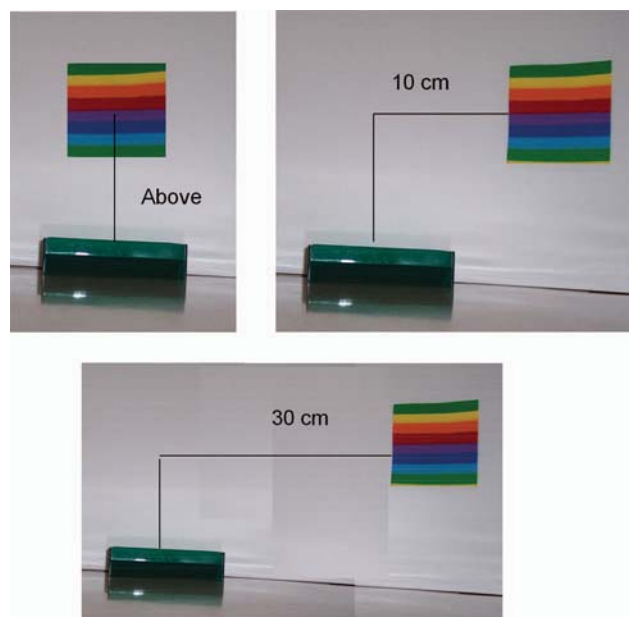


Figure 4. Spatial relationship between the goal (rewarded container) and the cue (a multicolored horizontally striped square) for the three groups of Experiment 2. The above group had the goal directly cued 10 cm above it. The near-10 and near-30 groups had their cues displaced 10 and 30 cm, respectively, to the right. See the online article for the color version of this figure.

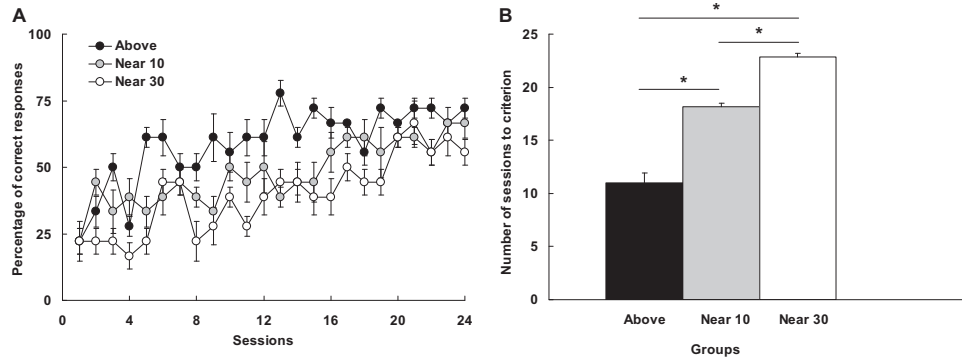


Figure 5. (A) Percentage of correct responses during acquisition (24 sessions) for the above ($n = 6$), near-10 ($n = 6$), and near-30 ($n = 6$) groups of Experiment 2. (B) Number of sessions to reach the learning criterion for each of the three groups. Means and confidence intervals ($\alpha = .05$) are plotted.

testing (a phase of six additional sessions). Toads were randomly assigned to one of two groups: near–near ($n = 7$) and near–far ($n = 7$). Toads of the near–near group were trained under a procedure in which the container with accessible water was surrounded by two visual cues: a red rhombus (10×10 cm) on the left and a multicolored horizontally striped square (10×10 cm) on the right when facing the cues (see Figure 6, left panel). The cues were placed on the wall 10 cm away and up from the edge of the reinforced container (same condition as the experimental group in Experiment 1). The position of the cues was inverted (i.e., rhombus on the right and square on the left) for three toads of this group for control purposes. Toads of the near–far group had one of the cues also positioned at 10 cm distant from the reinforced container (near cue) on the left, but the other one (far cue) was located at the same height but between the opposite container and the container to the right (see Figure 6, right panel). In this case, the near cue was the rhombus for four toads and the square for three of them for control purposes.

As the goal container was placed at a randomly changing goal point relative to the starting point, for each trial, the only way to reach the reinforced container was by using the visual cues as a reference. Other procedural details were the same as those described in Experiment 1. Once the acquisition criterion was reached, the overtraining and testing phase began. The probe trials were intended to determine the predictive value of each visual cue according to its position relative to the goal. Three types of probe trials were presented, two times each: (1) with both cues present, (2) with the cue on the right present, and (3) with the cue on the left present. Water reward was removed during the probe trials (toads were not reinforced). Depending on the container chosen, the response was classified as either correct or incorrect.

The percentage of correct responses per training session and body weight variation were recorded during acquisition. Unfortunately, for technical reasons, the weight variation data from four toads were lost. As such, the weight analysis includes only five

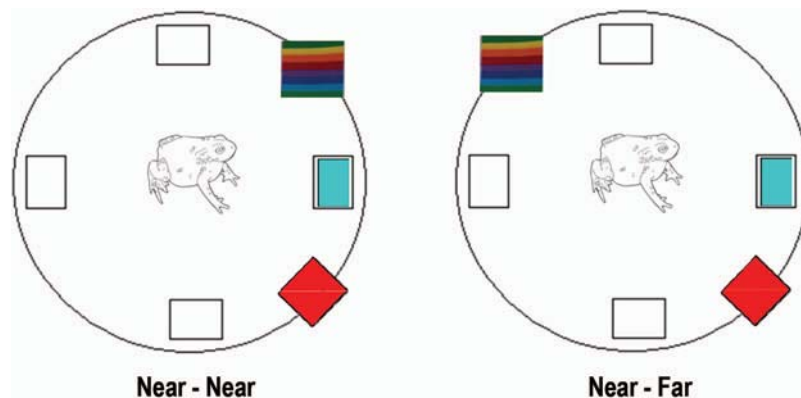


Figure 6. Schematic of the open field environment used for the toads of Experiment 3, showing the spatial relationship among the four plastic green water containers (placed North, West, East, and South), with one (light blue) containing accessible deionized water as reward, and the two possible positions of the visual cues on the walls. Group near–near had the two cues (a multicolored horizontally striped square on the left and a red rhombus on the right) relatively near the reinforced water container (left panel). Group near–far had one of the two cues (the multicolored horizontally striped square back on the left) relatively farther away from the goal, and the other cue (the red rhombus on the right) relatively near the goal (right panel). Toad drawing indicates the central start position used at the beginning of each trial. See the online article for the color version of this figure.

toads in each group. During the test trials, weight variation was not recorded as the toads did not have access to water.

Results and Discussion

Figure 7A shows the percentage of correct responses during training sessions for each group. A global analysis with a repeated measures ANOVA for acquisition revealed significant differences between groups, $F(1, 12) = 9.19, p < .05$, partial $\eta^2 = .43$, and across sessions, $F(15, 180) = 2.64, p < .005$, partial $\eta^2 = .18$, but no interaction effect ($p > .5$). This result suggests that toads in both groups learned the location of the goal, but with some differences in their performance. The two groups reached the acquisition criterion at different times: The near–near group took an average of 16 sessions to reach criterion, and near–far group took 18 sessions. To determine the source of this between-groups difference during the acquisition period, we analyzed learning rate (number of sessions to reach criterions; see Figure 7B). An ANOVA revealed significant differences, $F(1, 12) = 17.45, p < .005$, partial $\eta^2 = .59$, suggesting that the near–near group needed statistically fewer training sessions to locate the goal than the near–far group (16 sessions vs. 18 sessions, respectively). According to these results, the cues presented to the toads in the near–near group seem to be more effective in learning to locate the goal, allowing toads to learn the task and orient faster (i.e., these toads took fewer sessions to reach criterion).

Means of weight variation ($g/100\text{ g}$) were also recorded and were 1.16 ± 0.06 for the near–near group and 1.15 ± 0.06 for the near–far group.

During the overtraining period, the presentation of the probe trials did not affect performance in either group (group, session, and interaction factors; $ps > .5$). By analyzing data from the different testing conditions, we established the predictive value of each cue (see Figure 8). An ANOVA revealed nonsignificant differences between the groups' performance in each testing condition with two cues, $F(1, 12) = 0.16, p > .5$, or the cue available on the left (the near one for both groups), $F(1, 12) = 1.09, p > .1$. When the test involved the cue on the right—in this case, near for one group and far for the other—significant differences appeared, $F(1, 12) = 8.33, p < .05$, partial $\eta^2 = .41$. The results suggest that

both cues seemed to be equally salient for toads of the near–near group; they easily oriented using either of them alone. On the other hand, the cue on the right (far from the container) seemed to provide very little predictive value for toads of the near–far group. Hence, when only the right cue was available, toads found it difficult to locate the goal (in the latter case, their choice distribution did not differ from chance; groups vs. chance), $F(1, 12) = 6.25, p > .1$.

General Discussion

The goal of this study was to investigate cue-based navigation in an amphibian, the terrestrial toad, under laboratory conditions and provide novel information for comparisons with findings in other groups of animals. In our series of three experiments, we proved that an open field with intramaze visual cues can be used to test the importance of different visual cues for orientation by the toad.

Results of Experiment 1 revealed that toads can use proximal intramaze visual cues as beacons to orient in space in an open field situation. The association between the visual cues and the position of the reinforced container was easily established by toads in the experimental group after 24 training sessions. This learning strategy enabled toads to maximize water gain during exposure to the reinforcement.

From the results of Experiment 2, it may be concluded that one visual cue (independent of its distance to the goal [above or 10 or 30 cm away]) was sufficient to orient toward the goal. However, a graded effect of distance was observed during acquisition. Increasing distance with respect to the goal negatively affected the rate of acquisition; thus, the number of sessions necessary to reach the learning criterion was larger. As previously observed in mammals (Chamizo & Rodrigo, 2004), in the absence of any other relevant or causally related event, learning was possible even at relatively long distances between the cue and the goal. In addition, this experiment revealed for the first time that this phenomenon is also present in amphibians.

Finally, the results of Experiment 3 accord with those expected on the basis of previous observations in mammals, birds, fish, and invertebrates (see the introductory section): In amphibians, increasing the distance from visual cues to the goal also diminishes

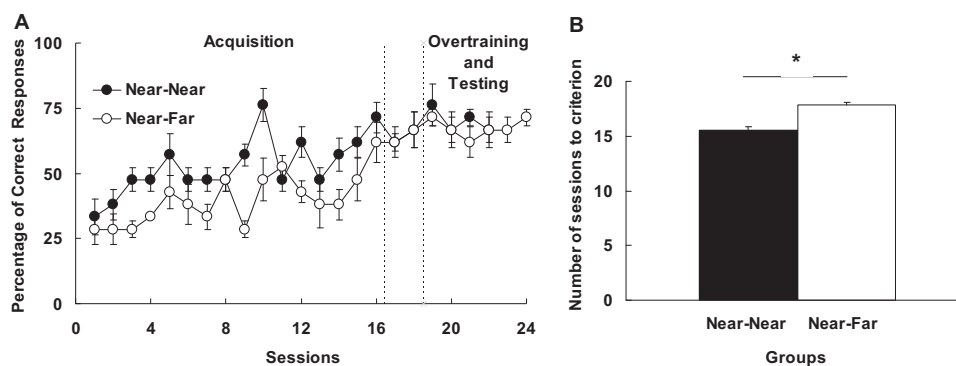


Figure 7. (A) Percentage of correct responses during acquisition (16 and 18 sessions) and overtraining and testing (six sessions) for the near–near ($n = 7$) and near–far ($n = 7$) groups of Experiment 3. (B) Number of sessions to reach the learning criterion for both groups of Experiment 3. Means and confidence intervals ($\alpha = .05$) are plotted.

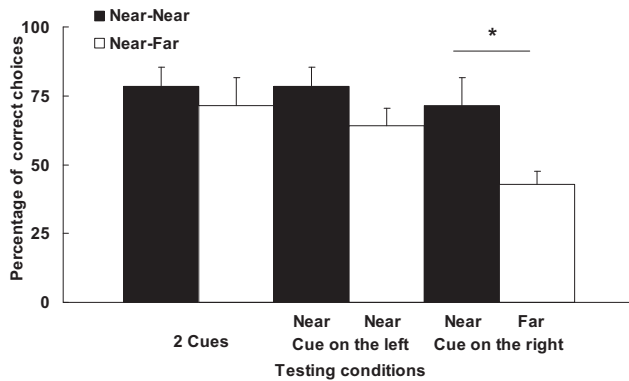


Figure 8. Percentage of correct choices during test trials (six tests, two each for the three test conditions for all toads) for the near–near and near–far groups of Experiment 3. Means and confidence intervals ($\alpha = .05$) are plotted.

their predictive value. This fact suggests that cues closer to the goal are considered more reliable for spatial orientation and “overshadow” more distant cues.

In this sense, our present results in toads confirm that proximity to the goal is determinant to establish cue relevance during spatial orientation, as predicted by the Rescorla and Wagner (1972) model. Moreover, the terrestrial toad relies mainly on proximal visual cues when orienting toward a water source in an open field situation. Thus, our data in toads contribute not only to understanding how amphibians process spatial information to locate a goal, but also shed light on this understudied vertebrate class, the first to adapt to terrestrial life. Overall, our current results in amphibians are in agreement with other data obtained from several nonmammal vertebrates, for example, fish and reptiles, suggesting that the ability to learn and rely on visual cues to orient toward and reach a goal has an ancient evolutionary origin.

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