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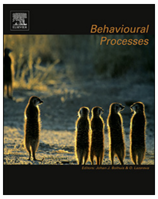

Rapid learning of a spatial memory task in a lacertid lizard (*Podarcis liolepis*)

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ABSTRACT

Mammals and birds are capable of navigating to a goal using learned map-like representations of space (i.e. place learning), but research assessing this navigational strategy in reptiles has produced inconclusive results, in part due to the use of procedures that do not take account of the peculiarities of reptilian behavior and physiology. Here I present a procedure suitable for testing spatial cognition that exploits a naturally evolved, ethologically relevant ability common to many lizards (i.e. refuge seeking behavior). The procedure requires lizards to learn the location of an open refuge inside a rectangular arena containing artificial refuges in every corner, using distal extramaze visual cues and with no local cues marking the location of the open refuge. The procedure probes the lizards' place learning ability and effectively rules out the use of egocentric and response-based strategies. The described procedure was successfully used to demonstrate place learning in a lacertid lizard (*Podarcis liolepis*). Over the course of two weeks of training both the latency to entering the open refuge and the number of corners visited in each trial decreased gradually, indicating that learning had taken place in over 60% of the lizards tested. These results confirm that, under certain circumstances, lizards are capable of navigating to a goal using a place learning strategy.

1. Introduction

Although reptiles are rarely used in learning and comparative cognition studies, there has been a recent surge of interest in reptilian cognitive abilities (e.g. Davis and Burghardt, 2012; Leal and Powell, 2012; Wilkinson and Huber, 2012; Matsubara et al., 2017; Qi et al., 2018; Whiting et al., 2018). Much of that interest has focused on spatial learning and memory (reviewed in Mueller et al., 2011). The focus on spatial learning is not surprising given the complex spatial demands that many reptiles face in their natural environment. Natural history observations and laboratory experiments clearly indicate that reptiles display a range of behaviors that may require the participation of cognitive mechanisms to solve spatial problems. Homing, exploratory behavior, and territoriality are common in species belonging to all major reptile groups (Stamps, 1977; Gregory et al., 1987; Chelazzi, 1992). The presence of such behaviors suggests that reptiles have the ability to learn about their environment and use this knowledge in fitness-enhancing ways.

Several recent studies have explicitly tested for spatial learning and memory in reptiles (e.g. LaDage et al., 2012; Mueller-Paul et al., 2012a, b; Noble et al., 2012). Others, while not specifically concerned with spatial learning, have used experimental designs that probe the ability of reptiles to solve problems with a spatial component (e.g. Amiel and Shine, 2012; Dayananda and Webb, 2017). However, despite this growing body of work, the evidence regarding spatial cognition in reptiles remains contentious. While some report that reptiles are as proficient at solving spatial problems as mammals or birds (e.g. López et al., 2001; Punzo, 2002; Salas et al., 2003; Noble et al., 2012), others find fundamental differences in the way that reptiles and other vertebrates navigate to reach a goal and even conclude that spatial memory as a navigation strategy may be absent in reptiles (Day et al., 1999, 2001; Day, 2003). Two different issues tend to be conflated. One is whether or not reptiles show any improvement in laboratory tasks requiring small-scale navigation to a rewarded goal. The other issue concerns the manner in which reptiles solve a problem with a spatial component, i.e. the nature of the representations that allow for efficient navigation. In particular, the possibility that reptiles may possess a hippocampus-dependent cognitive module similar to that supporting spatial learning and memory processes in other vertebrates has attracted much attention (Day et al., 2001, 2003; Jacobs and Schenk, 2003; Salas et al., 2003; LaDage et al., 2009; Holding et al., 2012; Striedter, 2016; Reiter et al., 2017).

Not surprisingly, most research on spatial learning and memory has been done with mammals and birds. This research has demonstrated that, in addition to several egocentrically-referenced mechanisms for orienting, mammals and birds are capable of place learning, i.e. forming map-like memory representations of allocentric space which are independent of the subject's position (O'Keefe and Nadel, 1978;

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Bingman, 1992). These map-like representations share a conserved neural substrate in the hippocampal formation (Striedter, 2016). Despite substantial structural differences, functionally similar hippocampi have evolved in all vertebrates (Butler and Hodos, 2005). However, the available evidence is insufficient to assess the extent to which different groups of vertebrates use similar or different strategies to solve spatial problems. Accurate performance in a spatial task can be subserved by a variety of mechanisms that are inherently nonspatial (e.g. beacons, response learning, trial-and-error searching, random paths). Therefore, the ability to solve tasks with a spatial component does not necessarily imply that animals use spatial learning and memory. For example, it has been suggested that turtles and lizards are more similar to fish than to mammals or birds in their tendency to use a response strategy rather than a spatial memory strategy to solve an eight-arm radial maze (Mueller-Paul et al., 2012a,b; see also Day et al., 2001).

Spatial learning has traditionally been studied by testing animals in maze tasks. Mazes of different shapes and various levels of complexity have been used to test spatial learning in reptiles (reviewed in Burghardt, 1977; Macphail, 1982; Morlock, 1989; Mueller et al., 2011). In the great majority of these studies the reinforcers used have almost invariably been either food or, in the case of lizards, access to a heat source or return to the home cage. Often, mazes and testing procedures have been adapted from those routinely used with rodents (e.g. radial maze, Morris water-maze), and may lack the ethological relevance called for in studies of reptilian cognition (Burghardt, 1977; Greenberg, 1995). As a result, reptiles may provide no evidence of learning (e.g. Bezzina et al., 2014), or need many trials to learn even relatively simple mazes (reviewed in Burghardt, 1977).

A frequently acknowledged function of spatial learning and memory is to provide information about the location of escape routes and predator refuges. Experimental studies have shown that familiarity with the local terrain improves the ability of many animals to escape from predators (e.g. fish: Aronson, 1971; Markel, 1994; rodents: Ambrose, 1972; Renner, 1988; Clarke et al., 1993). Responses to potential predators in reptiles involve a variety of avoidance behaviors, but for many species of lizards, access to burrows or crevices that provide shelter from potential predators may be the main form of defense against predation (Schall and Pianka, 1980; Greene, 1994; Cooper, 2015). Thus, an ability to track current location relative to the location of safe refuges could increase a lizard's chances of escaping from predators or aggressive conspecifics. Although scant, there is evidence that lizards learn the location of predator refuges within their home ranges (reviewed in Greene, 1994), and this ability may function in efficient predator evasion. For instance, fringe-toed lizards (*Uma notata*) respond to the presence of predator models by moving rapidly along straight paths towards burrows the entrances to which they cannot see directly (Adler and Phillips, 1985). Similarly, broad-headed skinks (*Eumeces laticeps*) escape from a human observer by making a direct run to the nearest available refuge (Cooper, 1997, 1998). In the same species, it has been shown that approach distance (the distance between the lizard and the observer when the lizard starts to flee) and the distance fled by the lizards are affected by the distance and angle to the nearest refuge, suggesting that lizards adjust their escape behavior based on their knowledge of the spatial location of predator refuges (Cooper, 1997).

Several recent studies have reported rapid learning of a naturalistic task requiring lizards to learn the location of artificial refuges inside an experimental arena (Amiel and Shine, 2012; Noble et al., 2012; Carazo et al., 2014; Dayananda and Webb, 2017). In these studies the lizards are given access to several visually indistinguishable refuges of which only one, designated the goal refuge, offers a suitable hiding place. Lizards are allowed to explore the experimental arena or are chased by the researcher until they enter the goal refuge, which is always found in the same location. To facilitate learning, lizards are given access to local (i.e. beacons) and/or distal, extra-maze cues. This experimental design takes advantage of the natural antipredator behavior of many lizards and therefore offers the potential to combine rigorous laboratory control with ethological validity. However, some studies using this design are not blind and lack appropriate controls for navigation using egocentrically referenced mechanisms and chemical trails. Therefore, they leave open the question as to what mechanisms allow lizards to navigate to a goal. In addition, as these studies have almost exclusively concerned two skinks (*Bassiana duperreyi* and *Eulamprus quoyii*) and one gecko (*Amalosia lesueurii*) from Australia, the available evidence has a strong geographic and taxonomic bias, which casts doubts on the generality of the findings.

The current study describes an improved procedure to assess spatial learning and memory in lizards. The described procedure was successfully used to demonstrate rapid learning of an escape task in a lacertid lizard. The experimental procedure requires that lizards locate and use an artificial shelter inside an experimental arena in response to a simulated predatory attack, using distal extramaze visual cues and with no local cues marking the location of the goal. The procedure specifically probes the lizards' place learning ability and effectively rules out the use of egocentric and response-based strategies. It provides a convenient alternative to the Morris water-maze and other similar procedures and may be suitable for testing spatial cognition in small terrestrial lizards.

2. Materials and methods

2.1. Subjects

The subjects in this experiment were 27 adult (9 females, 18 males) Spanish brown wall lizards, *Podarcis liolepis* (formerly *P. hispanica*), wild-caught by noosing around Valencia (Spain). *P. liolepis* is a small diurnal heliothermic lizard found mainly in rocky habitats throughout northeastern Spain and the Mediterranean coast of France (Renoult et al., 2010). Although information regarding their social behavior in the field is sparse, many small lacertids are territorial, have a polygynous mating system, and are routinely exposed to challenges that require the use of spatial learning to navigate within their natural environments (e.g. Strijbosch et al., 1983; Guillén-Salazar et al., 2007; Carazo et al., 2008). In particular, when approached by a potential predator, most lacertids will usually run to some sort of shelter, such as holes or crevices (Cooper, 2015). Field observations suggest that lizards remember the location of predator refuges within their home ranges and, in captivity, they readily enter artificial burrows. All lizards used in this study were held in captivity at least one month prior to commencing the learning trials to allow acclimation to laboratory conditions, including gentle handling by humans.

2.2. Lizard housing and maintenance

Lizards were housed individually in glass or Plexiglas terraria (25 × 50 × 30 cm) with soil as substrate, a rock for hiding, and a water dish. Terraria were kept in a temperature controlled room (range = 21–28 °C). A 40-W incandescent light bulb suspended above each terrarium was switched on for 14 h daily to provide light and a thermal gradient in which each lizard could select its body temperature. In addition, the terraria were exposed to indirect natural lighting, but not to direct sunlight, and a superimposed natural photoperiod from large west facing windows. The lizards were fed a diet of living arthropods in which mealworms (*Tenebrio molitor* larvae) predominated. Food items were occasionally dusted with a commercial vitamin and mineral supplement before being offered to the lizards. Water was available ad libitum. All testing took place in the same room where the subjects were housed.

2.3. Experimental apparatus

All tests were performed in an experimental glass-walled arena measuring 30 × 78.5 × 40 (high) cm (Fig. 1a). During testing the arena

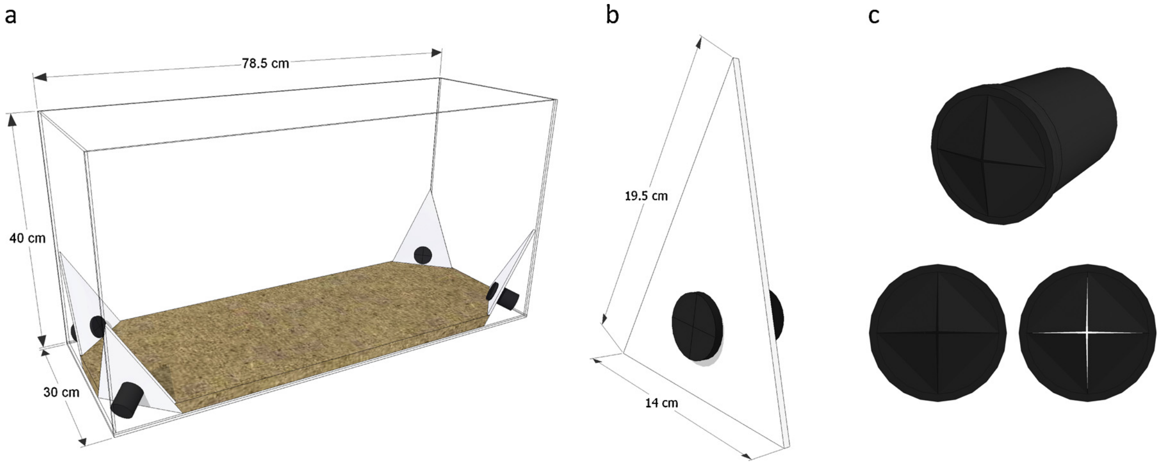

Fig. 1. Experimental arena and artificial refuges used in spatial learning experiment. a) Schematic drawing of the experimental arena showing the plates holding the canisters that acted as refuges on all four corners. b) Detail of a Plexiglas plate with canister in place; plates were glued to the glass walls of the arena with silicone. c) Schematic drawing of one of the photo film canisters used as artificial refuges for lizards; two lids are shown at the bottom, the one on the left is from a closed refuge, whereas the one on the right is from an open refuge (the two lids were visually indistinguishable when fastened onto the corresponding canisters).

was placed on a table, allowing an experimenter standing next to it to easily reach inside the arena. The arena remained in a fixed position in the testing room, allowing the lizards to navigate using positional cues external to the experimental arena. The arena had triangular pieces of opaque white Plexiglas (19.5 × 14 cm) in every corner. Each piece of Plexiglas had a round hole (33.5 mm in Ø) cut into it to accept an artificial predator refuge (Fig. 1b). Predator refuges consisted of empty 35 mm photographic film canisters. Three canisters had intact lids that prevented the lizards from entering the refuge, whereas a fourth had a circular opening (ca. 2.5 cm in Ø) cut into the lid allowing access to the canister. The entrance to both kinds of refuges was covered with a circular piece of pliable, opaque plastic screening fashioned from a light proof photographic black bag. The plastic screening was slit to create four flaps that joined in the middle, so that a lizard choosing the open canister could easily slide into it by pushing through the plastic flaps. Otherwise, the plastic flaps closed to obstruct the entrance making the two kinds of refuges (open and closed) visually indistinguishable (Fig. 1c). Once a lizard was inside the canister, the flaps closed to visually obstruct the exit. To facilitate learning, 2–3 open refuges were introduced in the holding terraria of the lizards during one week prior to testing. During that period, lizards were observed exploring and interacting with the refuges, and some of them actually used them as shelters. All tests were conducted between 13:00 and 18:00 h local time. Room temperature at the time of testing was 24–28 °C. To avoid disturbance to the lizards, their body temperature was not measured but it was likely higher than room temperature, as lizards in their home terraria could easily reach their preferred body temperature of 33–34 °C (Carretero et al., 2006).

2.4. Procedure

On the day preceding the experiment, all the participating lizards were allowed to freely explore the experimental arena with canisters in place for one hour (one open and three closed refuges). The location of the open refuge was held constant for each lizard but varied among lizards, i.e. for any one lizard the open refuge was consistently located in the same – randomly chosen – corner of the experimental arena. To begin a trial a lizard was gently taken from its home terrarium by hand and placed in the center of the arena. To minimize the possibility that they could use a fixed response strategy to locate the goal (e.g. run to the nearest wall, then turn left), the lizards were positioned in the center of the arena facing in a randomly chosen direction. There were no obvious intra-maze cues, but from inside the experimental arena the lizards had full sight of a room with diverse furnishings and large west-facing windows that could serve as distant (extra-maze) landmarks for orientation. Following an acclimation period of variable duration (10–70 s; see below), the experimenter approached the arena in a standardized way (i.e. at a slow pace, wearing similar bland clothing, and always from the same direction) and refuge seeking (escape) behavior was elicited by chasing the animals using the experimenter's hand with forefinger outstretched as a threatening stimulus. An attempt was made to keep lizards moving smoothly and continually by gently tapping them on the tail in a consistent way when they slowed or stopped. Although it may be argued that a human hand is not a natural lizard predator, this procedure has been successfully used in previous studies of antipredator behavior in reptiles (Herzog and Burghardt, 1986; Herzog et al., 1989a). In fact, it has been shown that a human hand is more effective in eliciting antipredator behavior from garter snakes than other, more realistic predator models (Herzog et al., 1989b). Similar procedures have been previously used to simulate predatory attacks in lacertids (Alkov and Crawford, 1965; Lecomte et al., 1993) as well as in other lizards (Paulissen, 2008; Dayananda and Webb, 2017).

In preliminary trials in which lizards were allowed 1 min before being chased, some of them remained motionless until the experimenter approached the arena. To discourage this behavior, in subsequent trials a random number generator was used to select the duration of the acclimation period (10–70 s) so that the lizards could not predict the waiting time until presentation of the threatening stimulus. The same person conducted all trials to eliminate potential inter-experimenter variability and the procedure was blind so that the person chasing the lizards did not know the location of the open refuges. A trial continued until the lizard entered the open refuge or until 60 s had elapsed. After a lizard entered the open refuge, it was returned to its home terrarium inside the removable canister and left undisturbed until it spontaneously left the canister.

Feces and other debris (e.g. pieces of shed skin) were removed after each trial and the substrate of the experimental arena, which consisted of loose sand, was changed at regular intervals. In addition, the sand substrate in the experimental arena was stirred and thoroughly mixed after every trial using a small garden rake to insure that any surface texture pattern or chemical trail left by a previous animal was disrupted. To control the possibility that the lizards may be cueing on olfactory cues emanating from the refuges, these were cleaned after each day's trials.

Each lizard was given four trials a day for 15 days. Dependent variables were the latency to entering the open refuge and the number of corners visited during each trial. The experimenter chasing the lizards used a stopwatch to record latency from the time the lizard started moving (whether spontaneously or in response to the experimenter's approach) and dictated the successive corners visited by each lizard to a second researcher located out of sight in the back of the testing room. Latencies were recorded to the nearest second. Criterion for scoring a visit to a corner was that any part of the lizard's body touched or came to within 3 cm of the Plexiglas plate in that corner. This criterion was easily applied given the proximity of the experimenter to the arena. The trials were not videotaped to avoid interference of the filming equipment with the experimenter that chased the lizards. If a lizard did not enter the open refuge within 1 min, the trial was terminated and was assigned a nominal latency score of 120 s.

On day 16, 10 randomly chosen lizards were given an additional probe trial. During probe trials the experimental arena was rotated 180° so that, for each lizard, the open refuge was now found in a location diametrically opposed to its assigned location during training trials. Probe trials were included to discard the possibility that the lizards may be relying on cues emanating from the goal to navigate to the open refuge.

2.5. Statistical analyses

Rather than using an arbitrary learning criterion, I used a decrease in the latency to enter the open refuge and in the number of corners visited to determine that learning had occurred. I predicted that, if lizards learn to locate the open refuge inside the experimental arena, they would require significantly less time to enter the refuge and would visit fewer corners as trials progressed. I evaluated these predictions by comparing the performance of lizards across trials in three ways. First, I used Page's nonparametric test for ordered alternatives (Meddis, 1984) to test whether latency and number of corners visited showed, as predicted, a monotonic decreasing trend. Second, I compared the mean latency in the first three days of testing (i.e. first 12 trials) to the mean latency in the last three days (i.e. last 12 trials) using a Wilcoxon paired-sample signed-rank test. Finally, the proportion of trials that resulted in a lizard entering the open refuge in day 1 versus day 15 was compared using a one-tailed Fisher exact probability test to determine whether the lizards showed any improvement across the training period. Since the a priori prediction is that latencies, corners visited and errors would decrease across trials, all statistical tests were one-tailed.

3. Results

Ten lizards were discarded because they failed to enter the open refuge at least once during the first 20 trials (several of these lizards simply froze and failed to move despite repeated prodding by the experimenter). The remaining 17 lizards (4 females, 13 males) learned to perform the task as shown by a significant reduction in the number of errors and the latency to enter the open refuge throughout the experiment (Figs. 2 and 3).

Both latency scores and the number of corners visited decreased gradually across the 15 days of testing [Z (latency) = 8.56, Z (corner) = 8.12, $p < 0.0001$ in both cases, Page's test for trends]. On average, the lizards took 101.81 s ± 6.60 (mean ± SEM) to locate and enter the open refuge during the first day of training. This latency decreased to only 42 s ± 3.77 by day 15. As expected, the average latency to entering the refuge during the first three days of testing was significantly larger than the corresponding latencies during days 13–15 ($Z = 3.47$, $p < 0.0003$, Wilcoxon signed-rank test). This result was further confirmed by comparing the proportion of errors (i.e. trials in which the lizard failed to enter the open refuge) at the beginning and at the end of the testing period: the proportion of trials resulting in a lizard entering an open refuge was greater on day 1 than on day 15 ($p < 0.0018$, Fisher's exact probability test). In fact, of 60 trials conducted on day 15, only in two trials two different lizards failed to enter the open refuge. During probe trials 8 out of 10 lizards tried to enter the refuge in the formerly correct locations as their first choice. The remaining two lizards did not try to enter any refuge during the allotted 60 s.

4. Discussion

Stereotypes and misconceptions regarding reptilian behavior and cognition are commonplace (Font et al., 2019). Despite abundant evidence to the contrary, there are still those who believe that reptiles are essentially instinct-driven machines, lacking in behavioral plasticity and incapable of the cognitive complexities often attributed to other vertebrates such as birds, mammals or fish (Butler and Cotterill, 2006; Vitti, 2013; Roth, 2015; Güntürkün and Bugnyar, 2016). However, a sparse but growing literature shows that many reptiles are in fact capable of most types of learning and that their cognitive abilities parallel or even surpass those of other vertebrates (Burghardt, 1977, 2013; Leal and Powell, 2012; Wilkinson and Huber, 2012). The results of the present work demonstrate that lizards learn to locate and enter a refuge in response to a simulated predation attempt, and thus add to the abundant evidence for sophisticated spatial cognition in a growing spectrum of reptilian species (Mueller et al., 2011).

The meager performance of many reptiles in learning experiments has been attributed, at least in part, to the use of inadequate procedures, stimuli or reinforcers (Burghardt, 1977; Macphail, 1982). Burghardt (1977) encouraged 'more behavioral and ecological familiarity …, coupled with imagination and a more sympathetic attitude' (p. 568) in the study of reptile learning, but this recommendation has gone largely unheeded judging from the relative scarcity of studies designed with reptiles in mind. For example, Foà et al. (2009; Beltrami et al., 2010) used a Morris water-maze to explore the role of the pineal eye in guiding navigation to a hidden platform in ruin lizards (*Podarcis sicula*). Although 8 out of 15 lizards reached a fairly modest learning criterion, ruin lizards are fully terrestrial, saxicolous lizards which rarely if ever enter the water, and therefore seem ill-equipped to solve a task that requires swimming to a submerged goal. The Morris water-maze was originally designed for work on rodents, which in general are good swimmers, but may not be as efficient to probe the spatial abilities of most lizards, excepting perhaps a few aquatic or semi-aquatic species.

The procedure described here does not require expensive equipment, is easily implemented, and yields rapid (1–2 weeks) learning in over 60% of tested individuals (the remaining lizards did not enter the open refuge in the first 20 trials, suggesting that their poor performance may be unrelated to spatial cognition). It resembles some devices commonly used in psychology laboratories to study spatial learning and memory in rodents such as the Barnes maze or the Morris water maze. However, the described procedure simulates an environmental challenge that many reptiles face in their natural environment (i.e. escaping from predators) and exploits a natural, ethologically relevant ability common to many small lizards (i.e. refuge seeking behavior). Although I used, for convenience, a rectangular arena (i.e. a large fish aquarium), the described procedure could be implemented using a square or a circular arena. This would have the added advantage of having all refuges equidistant from one another.

A reduction in latency scores (Fig. 2) does not necessarily indicate that lizards learned to locate the open refuge using a spatial strategy and could simply reflect habituation to the experimental procedure (Bezzina et al., 2014). However, the concurrent reduction in the number of corners visited over successive trials reinforces the conclusion that the lizards' performance was based on genuine place learning. In contrast to other tasks that require hundreds of trials (reviewed in Burghardt, 1977), learning using the described procedure was relatively rapid and was evident after just 4–8 trials. In comparison, using a modified Y-maze and return to home cage as reinforcement *P. liolepis* took hundreds of trials and several months of training to achieve a moderately stringent learning criterion (Font et al., unpublished).

After being released in the middle of the experimental arena lizards often spontaneously moved towards the open refuge, but did not enter

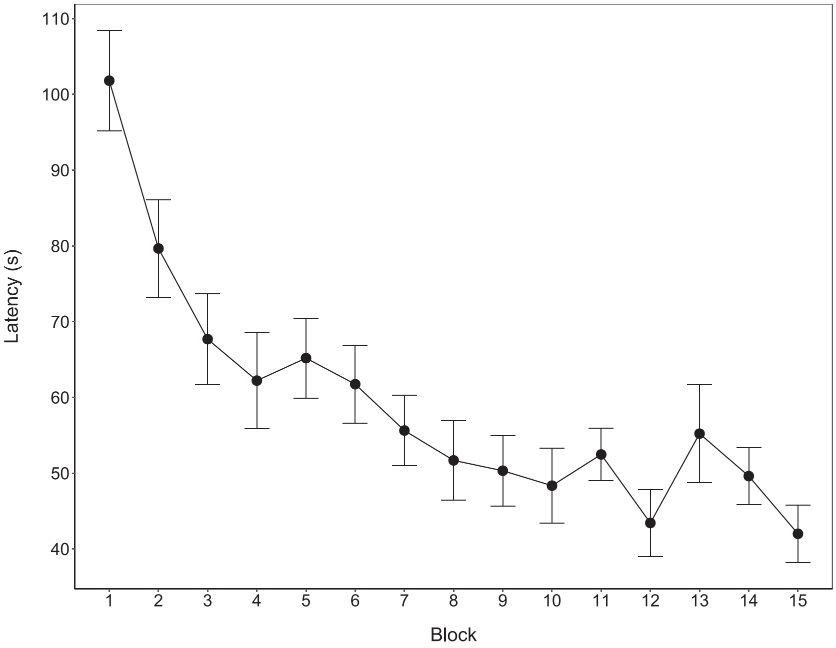

Fig. 2. Plot of latency data across 15 training blocks (4 trials/block). Mean (± 1 SEM) latency to entrance into refuge for the 17 lizards that entered the open refuge at least once during the first 20 trials.

it, instead remaining close to the refuge's entrance. When they were then approached by the experimenter's finger some jumped into the open canister, while others run around the arena before entering the open the refuge. Therefore, both the number of corners visited and the latency to entering the open refuge likely underestimate the spatial performance of the lizards. In particular, since lizards did not attempt to enter the refuges in many of the corners they visited during trials, the number of corners visited clearly overestimates the number of incorrect refuge choices. This is most evident during later trials, during which lizards approached several corners before entering the open refuge but made no attempt to enter the refuges in any of the corners they visited on their way to the open refuge. As a result, even though performance plateaued out after ca. block 9 in terms of both latency and number of corners visited, lizards were still approaching on average 3–4 corners before entering the open refuge (often the first one they visited). One possible reason why the lizards did not go straight into the open refuge during later trials may be lack of motivation, i.e. the lizards may have habituated to the mildly aversive stimulus used to motivate them to find the open refuge. Previous studies with lizards (Day et al., 1999; Paulissen, 2008) and snakes (Holtzman et al., 1999) have likewise noted that individuals that apparently have learned the location of the goal still revisit incorrect locations, even after extensive training.

The results of the present work largely agree with those of previous studies with lizards and with other reptiles. For example, LaDage et al.

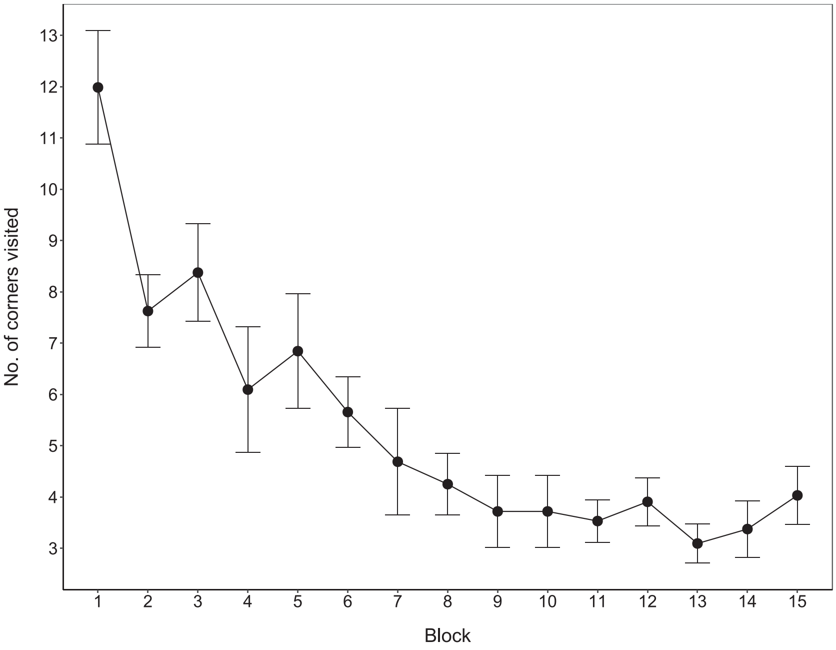

Fig. 3. Plot showing data on the number of corners visited across 15 training blocks (4 trials/block). Mean (± 1 SEM) number of corners visited for the 17 lizards that entered the open refuge at least once during the first 20 trials.

(2012) used a modified Barnes maze to train side-blotched lizards (*Uta stansburiana*) to navigate to an escape hole using return to home cage as reinforcer. The authors further showed that side-blotched lizards use a spatial memory strategy and rely on extra-maze cues to locate the goal hole (LaDage et al., 2012). Several recent studies have used procedures based on refuge seeking behavior to probe the learning abilities of three species of Australian lizards (Amiel and Shine, 2012; Noble et al., 2012; Carazo et al., 2014; Dayananda and Webb, 2017). These studies have consistently shown that lizards are capable of navigating to an artificial refuge that is found in a fixed location relative to intra- or extra-maze visual stimuli (for similar results with snakes see Holtzman et al., 1999; Stone et al., 2000). However, excepting Noble et al.'s (2012) study, which used three refuges, the remaining studies offered lizards a relatively simple choice between two refuges. Also, as the focus of most of those studies was not spatial learning per se, the evidence they provide is insufficient to determine whether the lizards' performance was based on spatial memory or on some alternative cognitive mechanism. As a result, conclusive demonstrations of unequivocal place learning in reptiles are still rare (López et al., 2000; Jacobs, 2003; Jacobs and Schenk, 2003; Mueller et al., 2011).

A recurrent issue in the comparative study of spatial cognition is the type of cognitive mechanism used by experimental subjects to locate a goal (e.g. Jacobs and Schenk, 2003). Animals use a variety of mechanisms to solve spatial problems and there is evidence of multiple spatial learning systems in vertebrates (reviewed in Shettleworth, 2010). For example, Punzo and Madragon (2002) showed that juveniles of three species of Australian *Ctenotus* are capable of learning to run a complex maze for a food reward using path integration. Mueller-Paul et al. (2012a) suggested that jeweled lizards (*Timon lepidus*) navigate a complex eight arm radial maze using a stereotyped turn-by-one arm response. In both these cases, lizards relied on an egocentric (self-centered) reference frame to locate the goal relative to their own position. In contrast, the procedure described here precludes the use of an egocentric frame of reference to find the open refuge since lizards are released facing in a random direction which is different from trial to trial.

In addition to egocentrically referenced mechanisms, many animals are capable of using map-like representations of spatial relationships (O'Keefe and Nadel, 1978; Nadel, 1991; Bingman, 1992; Clayton and Krebs, 1994). Cognitive maps were once thought to be the exclusive province of birds and mammals, but there is evidence that reptiles also use cognitive mapping strategies (Salas et al., 2003; Mueller-Paul et al., 2012b). In the present experiment there were no obvious visual cues inside the experimental arena, suggesting that lizards attend to extra-maze, rather than intra-maze stimuli to navigate to the open refuge. The results of probe trials further confirm that lizards were not locating the goal attending to cues (e.g. visual, olfactory) emanating from the open refuge. The experimental design used here thus rules out the possibility that the performance of lizards could be based on approach (or avoidance) of cues emanating from the goal, fixed, egocentrically referenced responses, or tracking of olfactory or other stimuli. Rather, the results suggest that lizards used map-like, relational strategies, by encoding the spatial relationships between the goal and an array of extra-maze, distant landmarks in an allocentric frame of reference.

It has been claimed that lizards need access to sun compass information that they detect through the pineal eye in order to successfully navigate to a goal (Foà et al., 2009). However, the lizards in the present experiment were indoors and even though they had access to natural illumination, the glass windows and the walls of the arena (also glass) likely blocked any polarized cues reaching the lizards. The performance of lizards indoors, in the absence of polarized light cues, suggests the existence of alternative (backup) mechanisms that allow lizards to navigate under different circumstances, as has been described for other vertebrates (e.g. Bingman and Cheng, 2005).

The results of the present study provide the first demonstration of allocentric place learning in lacertid lizards. A previous study with two lacertid species (*Acanthodactylus boskianus* and *A. scutellatus*) failed to find evidence of orientation to a single heated rock among seven unheated rocks (Day et al., 1999). The authors concluded that lizards do not use distal cues to navigate to a hot rock inside a circular arena and put forward the idea that the ability to navigate using distal visual cues may be an adaptation exclusive to birds and mammals. However, the distal cues in their experiments were located above the arena, rather than outside it. Results of the present experiment show that, when denied access to other cues (e.g. intra-maze stimuli), lizards can use distal extra-maze cues to successfully navigate to a goal. These results suggest that the purported spatial cognition gap between lizards and other vertebrates may be nonexistent. Additional studies with lizards in other families and with other reptiles will allow to evaluate the extent to which place learning is prevalent in this group.

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Declaration of Competing Interest

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