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Response-stereotypy in the jewelled lizard (*Timon lepidus*) in a radial-arm maze

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Abstract. Spatial cognition is an essential survival tool and a much studied area of cognition in mammals and birds. The radial-arm maze is a commonly used spatial cognition task, which requires an animal to move to a number of different locations and avoid revisits to previously rewarded ones. Although much is known about mammalian and avian radial maze behaviour, very little research has examined reptilian abilities in this setup. To date, only red-footed tortoises (*Geochelone carbonaria*) have been tested and mastered this task. The present study examined whether jewelled lizards (*Timon lepidus*) were able to learn a complex spatial task and investigated the cognitive mechanisms underlying this ability. Of three lizards trained in an eight-arm radial maze, one readily learned to navigate. Examination of its choice behaviour revealed the use of a turn-by-one arm response stereotypy. This shows, for the first time, that a lizard can successfully learn to navigate around a complex maze task. An understanding of the ability for and mechanisms of spatial cognition in reptiles is particularly important as reptiles do not possess those brain regions which have been found to determine spatial orientation in mammals and birds.

Keywords. lizard; radial-arm maze; reptile; navigation; spatial cognition; orientation

Introduction

The study of reptiles in paradigms comparable to those in which mammals and birds have been tested is important for our understanding of the evolution of cognition. Due to their evolutionary connection it is possible that the amniotic classes share common traits, but also, that differences may have evolved. This is particularly relevant in terms of navigation as reptiles do not possess a hippocampus or medial telencephalon, the regions which have been shown to be responsible for much of the navigation abilities in mammals and birds (O'Keefe and Nadel, 1978). One successful navigation method found in mammals and birds is the formation of cognitive maps by defining the goal in relation to a number of different landmarks (Arns, Sauvage and Steckler, 1999). Another orientation mechanism is response stereotypy, in which the goal is reached by following a learnt pattern of movements. This can either be a secondary process designed to reduce memory load and shorten travel distances (Dale and Innis, 1986) or it can be the primary orientation mechanism (Roitblat, Tham and Golub, 1982). Day, Crews, and Wilczynski (1999, 2001) found that three different species of lizard (*Acanthodactylus boskianus*, *A. scutellatus* and *Cnemidophorus inornatus*) did not use distal cues to solve a spatial maze task. The lizards might have used local cues of some kind, but this was not further investigated.

Early studies with a number of chelonian species (reviewed by Burghardt, 1977) showed that they have the ability to solve basic spatial problems. Subsequently, research has demonstrated some similarities between the mechanisms underlying reptilian navigation and that of mammals and birds (reviewed by Mueller, Wilkinson and Hall, 2011). Members of each class have shown both the ability to navigate based on spatial cues and navigation facilitation relying on response strategies. Wilkinson, Chan and Hall (2007) showed that a red-footed tortoise (*Geochelone carbonaria*) could successfully master an eight-arm radial maze (Olton and Samuelson, 1976). Tests ruled out the use of olfactory cues and suggested that the tortoise was using a cognitive map-like strategy. A follow-up study showed that, when required to navigate

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Table 1. Turn type usage of the jewelled lizard compared to chance. Calculation of standardised residuals: (observed N-expected N)/(√(expected N))

Turn type	Observed N	Expected N	Standardised residuals	sig	Performance compared to chance
1-arm	62	18.2	10.27	< .05	sig more
2-arm	8	18.2	-2.39	< .05	sig less
3-arm	3	18.2	-3.56	< .05	sig less
4-arm	0	18.2	-4.27	< .05	sig less

using impoverished distal cues, the tortoise navigated the maze by adopting a strategy of sequentially visiting adjoining arms (Wilkinson, Coward and Hall, 2009). When the tortoise was subsequently provided with a full array of distal cues it stopped using this strategy and apparently used the cues for orientation. This suggests that tortoises are able to use both cognitive map-like strategies without response stereotypy, and, when no cues are available, also pure response strategies. Thus, reptile spatial cognition might show higher levels of flexibility than that of mammals and birds.

However, it remains unclear whether the ability to master a complex radial maze is also present in reptiles other than chelonia. Therefore, the present study sought to investigate radial maze behaviour in squamata, specifically in the jewelled lizard (*Timon lepidus*). This species is ideal for examining the generality of potential reptilian abilities as it differs from the red-footed tortoise in terms of diet (largely insectivorous vs. largely frugivorous), habitat (temperate vs. tropical) and evolution (squamata vs. chelonia). Therefore, if maze learning is observed, it suggests that, amongst reptiles, this ability is not confined to the potentially distinct evolutionary line of chelonia (Zardoya and Meyer, 2001).

Materials and Methods

Three experimentally naïve, captive-bred male jewelled lizards (*Timon lepidus*) of 16 cm (Barney, sub-adult), 17 cm (Rocky) and 20 cm (Varanus) snout-to-vent length took part in the study. Two of them (Rocky and Varanus) were adults (aged >3 years), and one (Barney) was sub-adult (aged 2 years). The lizards were housed individually in plastic terraria and were provided with permanent access to water, shelter, and UV and heat lamps. Daytime and testing temperature was 28°C ± 2°C, night-time temperature 24°C ± 2°C, which is in accordance with husbandry standards (Janitzki, 2008). They were fed with gut-loaded mealworms in the experimental sessions and with crickets or hard-boiled chicken egg on rest days, with one day per week without food.

Apparatus

The apparatus was an eight-arm radial maze of the same dimensions as the one used by Wilkinson, Coward and Hall (2009) and Mueller-Paul et al. (2012). The maze had 14-cm-high opaque, plastic walls and the floor was covered with grip-ensuring rubber lining. The central area was an octagon with a diameter of 23 cm. Each arm was 18 cm long, 10 cm wide at the opening, and 25 cm wide at the back wall. An opaque, yellow, barrier, 2.5 cm high was fitted 4.5 cm from the back wall of each arm, to form a compartment in which food could be presented. The maze was covered by a 1.5 cm^2 wire mesh to prevent escape and was placed centrally in a 2.24 x 2.24 m windowless room lit with two 25 W fluorescent tubes. The distant walls contained features such as doors, sinks and shelves. A ceiling-mounted video camera that connected to a monitor in the adjacent room enabled online observation of the animals' behaviour without an observer present in the room.

Procedure

The experiment was run between July and November 2009. The animals were given one to four trials per day, five days a week between 9 am and 5 pm. Prior to the onset of the experiment, the lizards were habituated to the maze in 30-minute sessions. During this time they were allowed to explore the maze with food openly visible on the floor of each arm. Habituation was completed when an animal had eaten readily for three trials in a row.

Training: Throughout the training phase each of the eight arms was baited with one mealworm. Lizards were placed individually into the maze facing a randomly selected arm. They were allowed to move around the maze freely to collect the food rewards from the different arms. The rewards were initially presented in small bowls, but the lizards refused to feed from these. Accordingly, after 9 days of training, the use of bowls was discontinued, and the barriers described in the Apparatus section were inserted. The experiment was restarted and the results described below reflect the lizards' behaviour in the maze with the barriers.

In a room adjacent to the testing room the experimenter observed the order in which each animal entered the arms via a live video feed. An arm choice was counted when half the animal's body (without the tail) was inside the arm. The first visit to a previously unvisited arm was considered a correct choice. Any repeated visits to arms previously entered within a trial were classed as errors. The trial was completed when the lizard had visited eight arms regardless of whether the visits were to novel or previously visited arms. If the lizard had not made eight choices within 30 minutes the trial was ended and repeated later. If an animal had not moved for 15 minutes the trial was aborted; if it had not completed the trial but was still actively foraging after 30 minutes, the trial time was extended to 40 minutes. The criterion for success was a minimum of 40 completed trials and a success rate of at least 6 novel arms (5.3 = chance) visited during 18 out of the last 20 trials. The one individual to meet this criterion went on to two further tests.

Food odour test: This test examined whether the lizard followed the smell of the rewards and used this cue to select novel arms. Test trials were identical to training trials except that only four arms were baited. Two test trials were run. In the first, arms 1, 3, 5, and 7 and in the second, arms 2, 4, 6, and 8 were baited. Test sessions were run on two consecutive days and consisted of one test trial followed by one training trial.

Scent trail avoidance test: This test was designed to examine whether the lizard had learned to avoid previously visited arms on the basis of scent trails that it might leave. In this test all arms were baited, but to disperse olfactory cues the maze floor was flooded with a 5 mm layer of warm water. The lizard received four test trials on four consecutive days without any intermixed training trials.

Results

Training

All animals required only 3-6 trials to habituate to the apparatus. However, under training conditions two of the lizards often refused to move around the maze: One completed just 34% of the 19 trials it received; the other just 42% of the 27 trials it received. Training of these subjects was therefore discontinued. The remaining lizard, Barney, who completed 69% of his trials, reached the criterion of 18 out of 20 successful trials within the minimum of 40 completed trials. It is unclear why only one of the lizards worked in the task. Interestingly, of the three, he was the only sub-adult. It is possible that sub-adults of this species have a greater intrinsic tendency to explore than adults, or are more motivated for food (due to the nutritional needs of growth).

A one sample t-test revealed that Barney visited significantly more novel arms during the last 20 trials (mean 6.8 novel arms, SD = .76) than could be expected on the basis of chance (5.3 novel arms), t(15) = 9.235, p ≤ .001. However, Barney also performed significantly above chance during the first 20 trials (6.6 novel arms, SD = .88), t(15) = 7.373, p ≤ .001. This may reflect the natural hunting behaviour of jewelled lizards. That is, they may naturally hunt in a number of different locations, as has been shown in some related lacertid species (Huey & Pianka, 1981). Barney might simply have transferred this strategy to the experimental set-up. It is also possible that he had learned about acquiring food in the maze during the habituation phase or initial phase of training in which the food bowls were used.

Food odour test

To explore possible effects of food odour, a chi-square test examined whether, of the 12 novel arms he visited, Barney preferentially chose the baited (7 visits) over the un-baited (5 visits) arms. No significant difference, $\chi 2$ = 0.33, df = 1, n = 12, p > .05, was revealed. This suggests that Barney did not navigate the maze by following the odour of the reward.

Scent trail avoidance test

To examine whether Barney avoided arms that smelled of him a paired sample t-test compared his test performance in the flooded maze (mean 6.25 novel arms) with his performance in the last four training trials (mean 6.75 novel arms). No significant difference, t = -1.732, p = .18, was found. Furthermore, no behavioural differences were observed between movements in the dry versus the flooded maze. This suggests that Barney did not navigate the maze by avoiding his own scent trails.

Turning behaviour

Analysis of Barney's turning behaviour revealed that he preferentially entered adjoining arms sequentially. For each trial we scored the number of one-arm, two-arm, three-arm and four-arm turns in a row in one direction. A Chi-square test revealed a highly significant difference in the total number of turns of each type, pooled over the last 20 trials, $\chi 2$(df = 2, N = 95) = 53.64, p < .001. Analysis of the standardized residuals revealed that Barney used one-arm turns significantly more and all other turn types significantly less than expected by chance (table 1). The number of one-arm turns was positively correlated with success rate, r = .45, n= 40, p = .003.

A paired sample t-test analysing turning direction (regardless of the number of arms traversed) revealed that the lizard turned right by one arm (115 turns) significantly more often than left (49 turns), t(39) = 3.146, p = .003. Interestingly, the red-footed tortoise studied by Wilkinson, Coward and Hall (2009), did not show such a pattern, turning left and right equally.

Conclusions

The results of this experiment showed that a jewelled lizard can efficiently navigate an eight-arm radial maze. His performance, although slightly inferior to that observed in rats (Olton and Samuleson, 1976), appeared to be similar to that of the red-footed tortoise (Wilkinson, Coward and Hall, 2009) and of pigeons and in a comparable traditional radial maze setup (Bond et al., 1981).

Analysis of the lizard's choice behaviour revealed that he preferentially turned into the arm next to the one he had just left. This behaviour is similar to the response-based strategy seen in fish (Roitblat, Tham and Golub, 1982), and in a red-footed tortoise (Wilkinson, Coward and Hall, 2009) under conditions in which access to visual cues is restricted. We cannot rule out for this case, however, the possibility that the animal may be using visual cues to some extent such as has been demonstrated for other species (e.g., rats, Dale and Innis, 1986; humans, Aadland, Beatty and Maki, 1985; pigeons, Bond et al., 1981). However, as recent research conducted in this room (Mueller-Paul et al., in press) has shown that red-footed tortoises which had a similar head height to that of the lizards only had restricted access to the room cues in this setup, the likelihood is that the lizard disregarded these in favour of a primary response-based strategy. Future studies could clarify this by using maze rotation tests (Dale and Innis, 1986). Furthermore, the results match the finding by Day, Crews and Wilczynski (1999, 2001) who found that their lizards were solving spatial maze tasks by means other than distal cues integration.

The study of a single animal cannot inform about the general abilities and behaviour of that species; it can, however, tell us what the species is capable of. These results show for the first time that a lizard is capable of navigating a radial-arm maze and opens up an exciting avenue for future research.

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