DIPLOMARBEIT

Titel der Diplomarbeit

„ "How do Tortoises know where to go?" The use of cues in a T-maze task by sulcata tortoises (Geochelone sulcata) and leopard tortoises (Stigmochelys pardalis)"

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Inhalt

ABSTRACT Recent research with turtles and tortoises has shown that they are capable of using distal cues to find spatial locations and that they may have a map like representation of the environment to navigate. We wanted to investigate their discrimination abilities as well as orientation by training tortoises to use two different local cues to get to a distal goal. Therefore sulcata (*Geochelone sulcata*) and leopard tortoises (*Stigmochelys pardalis*) were trained to navigate in a T-maze. The cues were positioned at the top of the maze, the colour and/ or shape of the cues could be used to indicate the required response (turn left or turn right). Thus the tortoises were required to learn the difference between these two cues and use this information for finding the goal location. The results indicated that sulcata tortoises could learn to turn either leftward or rightward on the basis of the cue. The tortoises were then presented with a test which should show whether odour cues play a role or not. As expected scent cues were not used by them for solving the task. Following this the tortoises were presented with a final test in which the colour and shape cues were put in opposition with each other. Performance dropped to chance suggesting that both features of the cue were important to the tortoise when making the discrimination. This means their visual perception can discriminate different colours and shapes and they are able to associate features with directions.

INTRODUCTION

Spatial cognition is the ability to learn about and to remember locations (Healy, 2006). It is essential for survival and should therefore be observed in almost all species. It is particularly evident in animals that have to migrate over huge differences to find food or water, but it is necessary for all species that have to remember specific locations (Healy, 2006) in both small scale and large scale navigation. Small scale navigation has been investigated in animal groups such as birds (Cheng, Spetch, Kelly and Bingman, 2006) or invertebrates, for example bees (Lehrer, 1996) or crabs (Layne, Barnes and Duncan, 2003). Pigeons are well studied in large scale navigation, as they sometimes have to travel over far distances (Wallraff, 2005) as well as in small scale navigation. They use small scale navigation, for instance, to find their way back to specific feeding grounds. A review of Cheng, Spetch, Kelly and Bingman (2006) sums up the performance of pigeons in different spatial tasks. In all forms of navigation different cognitive processes might be included, for example the usage of a cognitive map, piloting with familiar landmarks or using specific cues to get to a goal (O'Keefe and Nadel, 1978; Griffin, 1955).

Using a cognitive map means defining a place by its spatial relationships to a number of landmarks. Therefore, even if some spatial cues become unavailable, the individual should still be able of accurate navigation based on the remaining cues (Nadel 1991; O'Keefe and Nadel 1978). The cognitive mapping system also allows the animals to use short cuts or start from a novel starting position and adopt novel routes to it (Bingman, 1992; Gallistel, 1990; Nadel, 1991; O'Keefe and Nadel, 1978; Thinus-Blanc, 1996). Hypothesis of piloting with familiar landmarks, compass usage and true navigation towards a specific goal were already described by Griffin (1955). In cases of guidance learning, compared to a cognitive map, the loss of a single cue is sufficient to disrupt the performance (O'Keefe and Nadel 1978). This means that these strategies are less flexible. The animal does not have a whole representation of the area, but still can navigate towards its goal. So the "routes" are a sum of guidance that can serve as landmarks to approach a location. Using distal cues or specific cues near the goal seems important (O'Keefe and Nadel, 1978; López, Rodríguez, Gómez, Vargas, Broglio and Salas, 2000), depending on what is needed in the moment of action.

The cognitive mapping system as well as using landmarks and cues for orientation require forms of perception, discrimination abilities, memory and learning (O'Keefe and Nadel, 1978). Many different apparatuses, instruments and training methods were established to investigate these assumptions (for a full review see Burghardt, 1977). There are many different forms of mazes; the simplest is a straight runway where the animal can only move from one end to the other. Others may include several choice points; water mazes, submerged or partly filled with water as well as elevated or enclosed (Burghardt, 1977) and some are radial with different numbers of arms. The radial maze is a classic task to examine spatial orientation and working memory in animals (Olton and Samuelson, 1976; Wilkinson and Huber, 2012). A simpler form, also used in the current study, is the T-maze, sometimes also called Y-shaped maze. It involves only one choice point and can be well used for discrimination studies (Burghardt, 1977). Other examples are the studies of Olton and his colleagues, tested the performance of rats in an eight-arm radial maze and after that in a seventeen-arm radial maze (Olton and Samuelson, 1976; Olton, 1977; Olton, Collison and Wertz, 1977). The rats had to learn to visit only the arms of the maze which were baited with food, always starting in the middle. They learned quickly and did not use simple strategies, such as visiting one arm after another, also were no odour cues involved in their decisions. Only extra–maze cues seemed important to them. Therefore they formed a map-like representation of the cues in their environment and used them for navigation. Mazes were established to study forms of spatial navigation, discrimination problems, escape behaviour and learning. The process where organisms respond more frequently to a stimulus correlated with reinforcement than to another stimulus correlated with non-reinforcement is called discrimination (Terrace, 1963). Studies showed that it only works by using both stimuli, one reinforced and one not. The reinforced stimulus and the non-reinforced stimulus must be alternated during the procedure to get the desired response (Terrace, 1963). Animals which are able to discriminate between different cues with different features show that there must lay a function behind it (Watanabe, Lea and Dittrich, 1993) and their abilities give information about the visual system. Much work about visual discrimination was done with birds, especially pigeons, for example by Von Fersen and Lea (1990). Birds have very good discrimination abilities as they need them in their everyday life to find food, look for birds of prey and during flying (Bruce, 2003; Harley, 1972) also because they are a highly mobile species.

In the last couple of years, cognitive studies have largely focused on the capabilities of mammals, birds and fish (Bond, Cook and Lamb, 1981; López, Rodríguez, , Gómez, Vargas, Broglio and Salas, 2000; Vargas, López, Salas and Thinus-Blanc, 2004). However, to gain a better understanding of vertebrate cognition reptiles should be more considered in cognitive studies (Mueller, Wilkinson and Hall, 2011; Mueller-Paul, Wilkinson, Hall and Huber, 2012). The group of reptiles was studied little and not always accurately in the last centuries, although they could provide information not only on the spatial learning and memory strategies of living reptiles but also insights in the evolutionary and adaptive aspects of spatial capacities in all vertebrates (López, Gómez, Rodríguez, Broglio, Vargas and Salas 2001) as they all belong to the group of amniotes (mammals, birds, reptiles). Reptiles though split from the rest of the amniotes 280 million years ago (Macphail, 1982) and it would not be surprising if they evolved different strategies of spatial navigation and cognition. They do possess different brain structures as mammals, birds and fish, for instance they lack a hippocampus, which is essential in spatial navigation in mammals (O'Keefe and Nadel, 1978). In O'Keefe and Nadel (1978) it is postulated that in mammals the hippocampus forms the cognitive map and is involved in landmark usage, whereas beacon homing is processed in a different region in the brain. This has been confirmed by lesioning experiments in rodents. Lesions in the hippocampal area let the animals still use beacon homing but not landmarks for orientation (Save and Poucet, 2000; Poucet, Lenck-Santini and Save, 2003). In the review of Cheng, Spetch, Kelly and Bingman (2006) the importance of the avian hippocampus in spatial orientation is outpointed. The hippocampal formation in avian spatial cognition plays a role in the recognition of goal locations. Researchers assume that the medial cortex might have similar functions in reptiles (Rodriguez et al., 2002). To investigate this hypothesis lesion experiments in the medial cortex of terrapins were run by López, Vargas, Gómez, and Salas (2003). First the animals were trained to navigate in an open field task by using distal cues. After the lesions they were not able to use these distal cues anymore, but when retrained on the task, the lesioned animals learned to navigate to the goal again. This time they seemed to orientate on local cues, but performed at the same level as before. These behaviours are very similar to those observed in mammals and profound the assumption that the medial cortex has similar functions to the mammalian and avian hippocampus (López, Vargas, Gómez and Salas, 2003). It suggests that chelonians (turtles, tortoises and terrapins) have the ability to form a cognitive map (López, Rodríguez, Gómez, Vargas, Broglio and Salas, 2000) and navigate with extra maze cues. Studies of López et al (2000) revealed that turtles trained in a four arm maze on a place procedure, where food rewarded a specific place in the maze, seemed to use a map-like representation of the extramaze cues provided for solving the task. Turtles trained in the place procedure managed the task from different starting points with accuracy even though they had partial loss of environmental information. The second group of *Pseudemys scripta* was trained in a cue procedure, where an intramaze visual cue could be used as a beacon to find the goal arm and no further landmarks. Also this task was managed with accuracy. Mueller–Paul, Wilkinson, Hall and Huber (2012) could also show that tortoises changed their strategy depending on the cues available and signs of flexible behaviour in terms of orientation.

Early research into reptile cognition started more than a century ago with Yerkes in 1901. It was the first study on learning in reptiles and it included the usage of a maze (Burghardt, 1977). He investigated maze learning abilities in the speckled turtle, *Clemmys guttata*, followed by Tinklepaugh (1932) for the common wood turtle, *Clemmys insculpta.* Both showed that the turtles could learn to navigate in a multiunit maze. A few other colleagues then followed examining different abilities of chelonians, for example, Seidman (1949), who did a comparison of terrapins and newts in a simple T-maze task and reversal learning. He showed that terrapins learn better compared to newts and he proposed that it is because newts lack a neopallium. Later on, Kirk and Bittermann (1963) showed early attempts of turtles solving habit reversal tasks in a T-maze. Further studies of Holmes and Bittermann (1966) confirmed these findings about the ability for reversal learning in the T-maze in turtles.

In the field of discrimination learning in chelonians, work was largely focused on visual discrimination abilities in turtles (Davis and Burghardt, 2007). Visual abilities were trained in numerous respondent and instrumental learning tasks (Kirk and Bitterman, 1963; Bitterman, 1964), starting with the study by Yerkes (1904) about the reluctance of box turtles to walk over an edge. The phenomenon has been termed "visual cliff" and, since then, the method used in that study has become very popular. It has been applied in comparative studies with different species, including rats, chicks, and also human infants (Walk and Gibson, 1961; Routtenberg and Glickman, 1964; Burghardt, 1977). However, a more common way to study discrimination abilities, is the usage of training the animals on different stimuli and let them choose. Studies investigated if turtles could discriminate horizontal and vertical lines, and parallel lines of different width (Casteel, 1911). Other studies tested the discrimination of colours, brightness, form, size (Kuroda, 1933; Wojtusiak, 1933, 1934), hues and greys with paper and lights (Quaranta, 1952) or monochromatic spectral light thresholds (Armington, 1954; Muntz and Sokol, 1967; Muntz and Northmore, 1968). A review of early discrimination studies is given by Burghardt (1977). Studies revealed that some colours are more easily discriminated by turtles and tortoises than others; namely, they were found to be less sensitive at the blue end of the spectrum (Burghardt, 1977).

More recent work carried out by Wilkinson, Chan and Hall (2007) focused on working with a red-footed tortoise (*Geochelone carbonaria*) in an eight-arm radial maze. In the study with the red-footed tortoise "Moses", a single individual was trained to navigate in an eight-arm radial maze to find baited arms. The tortoise performed reliably above chance, visiting the baited arms rather than the ones that were seen before. In this study the maze was open to the room. Odour cues did not affect the tortoise's performance in the maze; instead the animal showed behaviours comparable to those of mammals (Wilkinson, Chan and Hall 2007). A follow up study was done by Wilkinson, Coward and Hall (2009) to test which of the cues were important to the animal for navigation. For this, they surrounded the radial maze with a curtain and fixed only 4 specific cues which differed in colour and shape. They were easy to manipulate once the tortoise had learned the task. Unexpectedly, the tortoise did not appear to use the cues provided and instead started to use a response based strategy, visiting the arm adjacent to the one that it had left. When the tortoise was trained again with all the extra maze cues of the room, without a curtain around the maze, it started to use distal cues again (Wilkinson, Coward and Hall, 2009). Findings in other reptile orders enforced the general view that this animal group has a general ability of solving spatial and discrimination tasks. Maze studies varied greatly regarding the form of mazes, methods and reinforcement among different reptile orders and therefore comparative conclusions are difficult to make. They might allow for conclusions about sensory factors and life history rather than brain complexity (Burghardt, 1977).

Chelonians are good study species as they work hard for little rewards over a long period of time (Burghardt, 1977; Kramer, 1989). Some species are readily available and adapted well to perform in captivity and live long. Turtles seem to be highly visual and have the ability to discriminate colours and shapes (Arnold and Neumeyer, 1987). (For a review of early studies in chelonians see Burghardt (1977). The African spurred tortoise *Geochelone sulcata* is an interesting species to study spatial navigation as it digs its own burrows. These burrows provide protection from very high temperatures during the day time, from humidity and also against the really cold nights in the desert (Gurley, 2002). African spurred tortoises have to face a temperature gradient from 6°C during nights up to 48°C the shadow during day time. Usually the animals always return to these burrows. During the wet winter seasons, they start digging an average of three different burrows which they use during summer time, when it is really hot and dry (Gurley, 2002). This circumstance requires some form of spatial orientation to find the way back. Spatial memory must be involved in this process as well as good navigation through the desert, although it is not known which form of navigation. In general, sulcata tortoises live in the Sahel Zone and tropical sub desert steppe of northern Africa and are the 3rd largest land living tortoises. Their average weight is about 80kg for males, 50kg for females and a characteristical feature is their sandy coloured skin plus big spurs on their limbs (Grub, 1971). Leopard tortoises however do not dig burrows at all. Usually they take different plants as shelters and favour more semi-arid, thorny to grassland habitats throughout savannahs of Africa from Sudan to the southern Cape. They are also a large species, with a carapace length of more than 50cm and a weight of 30kg. *Stigmochelys pardalis* feeds on a wide range of different plants, depending on the season and their availability (Milton, 1992). Their diet is largely vegetarian, comprising a wide variety of plant items, also shown in previous studies of Loveridge and Williams (1957) or Milton (1992).

Comparing these two species is particularly interesting as both come from semi-arid to arid zones of Africa, they inhabit similar areas, both are herbivores and do not hibernate at all, but still their ecology differs in terms of shelter use and diet (Gurley, 2002; Loveridge and Williams, 1957). It would be interesting to see whether sulcata tortoises, which are natural diggers and use the same burrows all the time, would perform better in a spatial search task than leopard tortoises which usually do not have to orientate that much in their natural habitat. Leopard tortoises instead have some food preferences. When specific plants are available, they actively look for them. This could be associated with improved abilities and behaviours in terms of discrimination, like looking for different colours of flowers or maybe looking for different cues in their habitat. The difference in the ecology of these two species could involve different discrimination and cognitive abilities. For instance, *Geochelone sulcata* may navigate better, whereas leopard tortoises may have better discrimination abilities when it comes to colours or shapes, or even smell.

The current study was conducted to improve our knowledge about navigation and discrimination skills of tortoises. Two intra maze cues, differing in colour and shape were presented. These cues were indicative of a specific distal reward location. Thus, one specific cue must be remembered, recognized and associated with the right location. Previous studies of Wilkinson, Chan and Hall (2007) and Wilkinson, Coward and Hall (2009) provide evidence that tortoises are capable of using distal cues for orientation. However, the position of these cues could be used to predict the position of food. In the current setup only the shape and colour of the stimulus could be used to learn the position of the reward. Furthermore this study investigated this ability in two species, sulcata (*Geochelone sulcata*) and leopard tortoises (*Stigmochelys pardalis*). The comparison of two species can increase our knowledge about tortoise learning and the roles that ecology can have upon these processes.

MATERIALS AND METHODS

Subjects:

A total of eight juvenile sulcata tortoises (*Geochelone sulcata*), four males and four females, and two adult leopard tortoises (*Stygmochelis pardalis*), one male and one female, took part in this experiment. All individuals of *Geochelone sulcata* were between 7 and 15 years old. The exact age of the leopard tortoises is unknown. Both species were kept in an enclosure at the Parrot Zoo, Friskney, UK, which is a registered animal sanctuary. The indoor part of their enclosure, 10m^2, had a heated floor with a temperature gradient from 20°C up to 28°C and a spot lamp of over 30°C. During the night the temperatures where lowered to about 12°C. Outside, the animals had access to a 65m^2 grassy area. They were fed a rich and balanced diet consisting of hay, grass, lettuce, carrots and celery. The animals were not food deprived but were rewarded with favoured foods.

Materials – T-maze

For the experimental set up a T-shaped maze was built of wooden panels with a total length of 244cm, a total width of 244cm and a height of 61cm (Figure 1). The inner area of the maze contained a surface of 3.48m^2. Walls of the maze were painted white and the maze floor was covered with grey kitchen liners made of plastic which could be cleaned easily. The left and the right arm of the maze protruded 81cm on each side and had a width of 92cm (Figure 1). A plastic reward bowl was placed at the end of each arm. The maze was set up in a test room with a width of 3.5m and a length of 8m. All the walls of the room surrounding the maze were white to discourage the use of extramaze cues. The room temperature varied between 19°C and 25°C. Natural daylight from windows on two sides of the room served as source for light. For this task two differently coloured and shaped stimuli appeared in each session, with only one cue being presented per trial. Therefore two conditions, distinguished by the cues, were created for the individuals (Figure 1). The rewarded side depended on the cue presented and the animal. The stimuli for the Pilot Study were a red circle with a diameter of 30cm and a blue triangle with a total length of 30cm made of coloured, laminated sheets of paper (Figure 1).

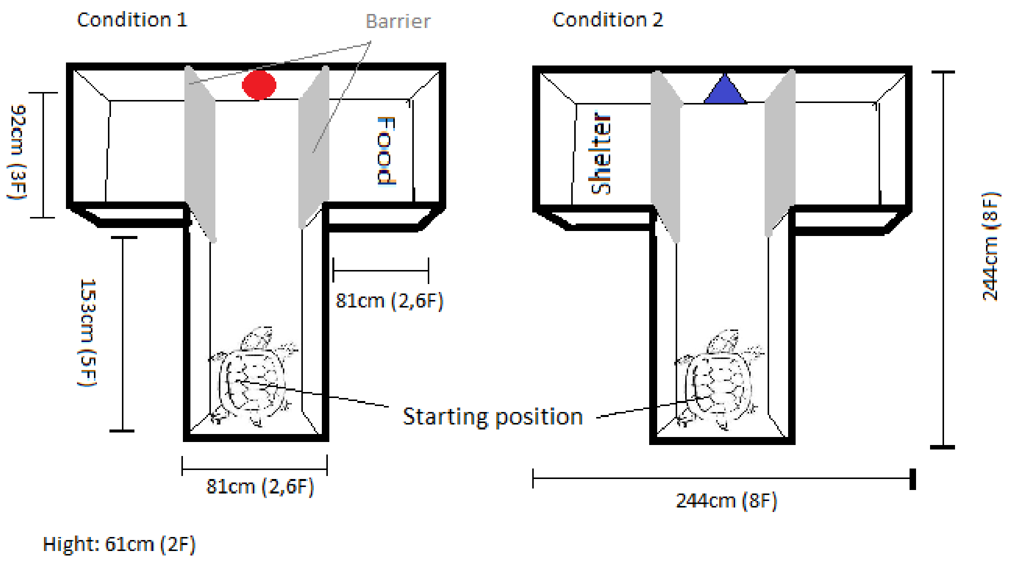

Figure 1: The T-maze was 244cm long and 81cm wide so that all tortoises could fit in the maze and still had enough space to move around easily. The height was 61cm and should prevent the usage of external cues. In the training and testing conditions opaque barriers hid the rewards. The picture above shows set up 1 for the pilot study, with the red circle in Condition 1, as well as Condition 2 with the blue triangle as navigational cue. All individuals were trained on both conditions in a randomised order, one trial presenting Condition 1 and another trial presenting Condition 2.

For the training two additional cues were produced, a yellow square with a total width and length of 30cm and a black star that was cut out of a circle with a diameter of 30cm. During the training and the testing sessions rewards were hidden by opaque barriers made of plastic. These barriers were placed at the entrance of each arm of the maze (Figure 2), hanging down from the top of the maze and lose at the end so the tortoises could walk through easily by pushing against them.

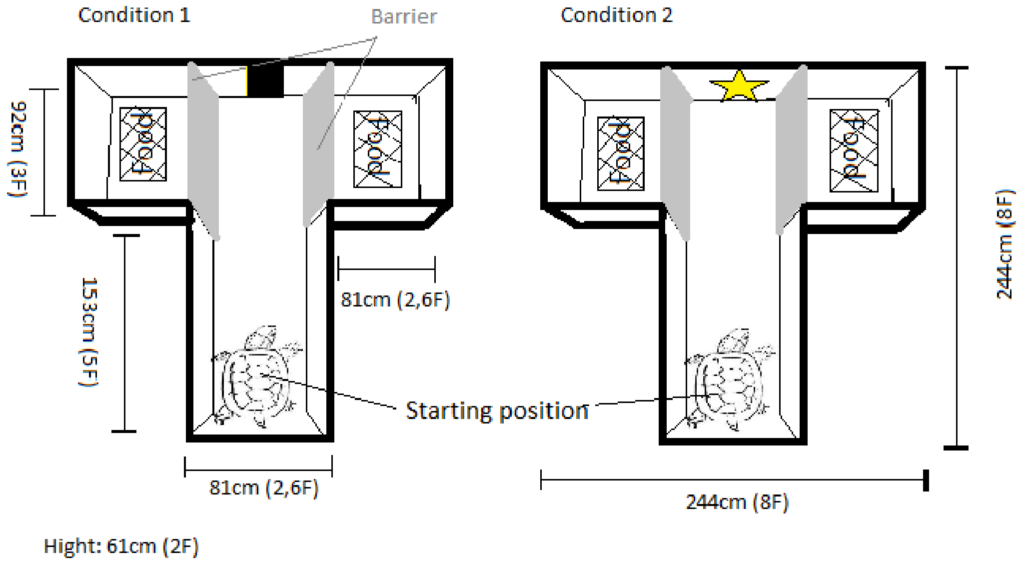

Figure 2: During the training, set up 2 new cues were presented to the tortoises. Condition 1 meant the black star and Condition 2 the yellow square as navigational cue. Each session contained three trials with Condition 1 and three trials of Condition 2 in a random chosen order. As rewards only foods were presented to keep their motivation.

To prevent odour cues, the plastic liners on the floor were cleaned after every single trial and also the side walls of the maze if necessary. As rewards, favoured food, such as tomatoes, strawberries mixed with salads, carrots, pear and sometimes melon were presented in the plastic bowl. In the pilot study a shelter made of a wired mash covered with branches was used as a different reward.

Procedure

The experiments ran from April until mid of August 2012. The animals were trained one to six trials a day, depending on their motivation and weather conditions. First the animals were habituated to the new experimental set up, then the training phase started. After the learning criterion was reached (see chapter "Training") the animals were tested in the T-maze.

Habituation

Before the training could start the tortoises were habituated to the maze to ensure that they moved around readily. For this, every individual received two sessions with openly visible food items spread around the maze. The habituation was considered complete when the tortoises ate and showed no signs of escape behaviour for two sessions in a row. Between the sessions the individuals were brought back to their enclosure for at least an hour. Animals that did not move readily or did not eat while staying in the maze received more sessions to ensure that they were comfortable with the situation. Each session took 30 min.

Pilot study

After the habituation a pilot study was conducted to find out whether food or shelter was more rewarding to the tortoises. Each individual received 20 trials. A red circle and a blue triangle (Figure 1) served as stimuli, with one of the cues being rewarded with a shelter; the other one was rewarded with food. This was counterbalanced across the animals. For some tortoises the red circle was rewarded with the shelter, whereas for others it was rewarded with food, the same was true for the blue triangle. The sequence of the cues presented in a session was chosen randomly. The pilot study showed that a shelter was not rewarding for the animals and therefore it could not be used for further training. Most of the tortoises did not go into the shelter but tried to eat the branches it was made of instead of hiding in it. Whereas the shelter was not used, food was favoured by all animals. Therefore the experiment was adapted and only food was used as a reward.

Training

At the start of each trial the tortoises were individually placed into the maze at the starting position (Figure 1) so that they were facing the cue in front of them. In each trial they were presented with either the yellow square or the black star and were rewarded with food on one or the other side (Figure 2). On the unrewarded arm of the maze food items were placed under a transparent bowl so that they had no access to it. This was implemented to reduce the likelihood that they simply followed the smell of food. The experimenter was behind the starting position, observing and documenting the tortoises' behaviour from above. The tortoises could not see her. Throughout the training, in each trial the animals had to walk towards the cue and then either turn left or right depending on which cue was presented. Visual barriers at the beginning of each arm of the T-maze (Figure 2) stopped the subjects from seeing whether the reward was available before making a choice. A choice was counted when they were half way through the barrier. Trials were terminated and rerun later if the animals failed to make a decision within 8 minutes. To reduce the impact of any side, reward and colour biases, these were counterbalanced across the individuals. Daily training sessions included the use of correction trials. If the tortoises chose the incorrect side, they were removed from the apparatus and the trial was repeated until the correct choice was made. Only the first choice was recorded for data analysis. All animals had to finish 17 sessions, so they had 102 training trials in total. The learning criterion was set at a performance of 14 trials correct out of 18.

Testing

Scent – Test

After the individuals reached the learning criterion a scent test was performed to examine whether the tortoises used odour cues from the food to solve the task. For this, the same set up and procedure as in the training was used, with the exception that all rewards were removed from the T-maze (Figure 3) and the animals got rewarded after they made a choice. The scent test was made up of two sessions, each session containing six testing trials. At the beginning, the tortoises were placed at the starting position and then had to complete all trials without getting their rewards inside the maze. When the subjects made a right choice, they were taken out of the maze and received a reward immediately. Again they had 8 minutes to make a choice before the testing trials were terminated. Clean, empty plastic bowls stayed in the maze to ensure that nothing except of the food changed in the testing condition.

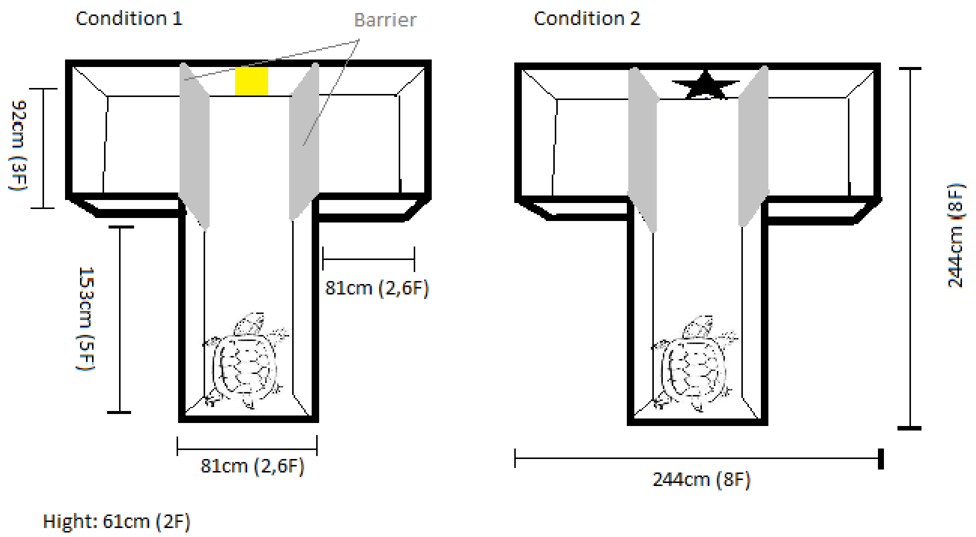

Figure 3: Set up 3 was presented during the Smell test. It contained the same cues and both Conditions of set up 2, but no food rewards were placed in the plastic bowls in the maze so that the tortoises could not follow any scent cues. The animals got rewarded immediately outside the maze after they made their choice.

Feature – Test

To test whether the tortoises learned about the colour or about the shape of the cues we introduced the Feature test. The Feature test presented the cues in conflict with one another. Thus instead of the black star they were presented a yellow star, and a black square instead of a yellow one (Figure 4), to see if the animals had learned predominantly about the colour or shape of the stimulus and predict the choice of direction. Each test session contained six training trials and two test trials. Four sessions were conducted, resulting in eight test trials. During the test trials the new cues were presented but the animals received no differential feedback after their choice. This method was used to prevent the tortoises from learning about the new cues.

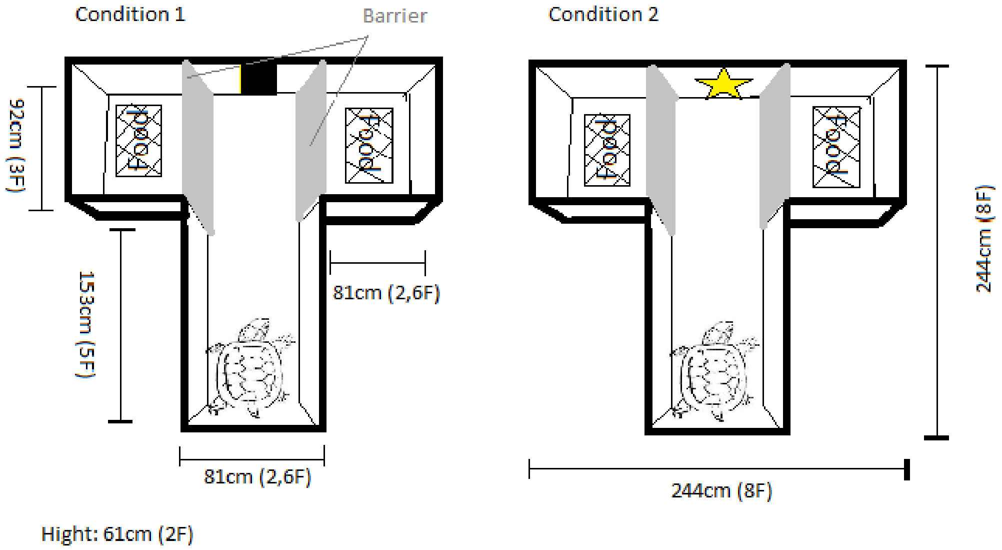

Figure 4: The maze in set up 4, for the Feature test, contained new cues and no accessible food during the testing trials, that the animals did not get rewarded for their choices. These two testing trials were put within six training trials (set up 3).

RESULTS

Out of eight sulcata tortoises only four were able to finish all 17 training sessions due to motivational problems and illness. They were therefore removed from the experiment. This left six tortoises; four sulcata and two leopard tortoises.

Training

Over two months of training each animal received 102 training trials. The results of the training sessions are shown in Table 1. In total, two out of four sulcata tortoises (Bob and Squeeze) reached the learning criterion. The other participating tortoises performed at varying levels but did not reach the learning criterion. The two tortoises that did reach learning criterion did so after 12 and 16 sessions, respectively.

Table 1: Number of all correct trials in 17 sessions of each individual. The grey marked numbers show the sessions where the tortoises reached the learning criterion and how many trials they got right out of 6: Bob got 15 right out of 18 trials and Squeeze 14 out of 18. Dawn, 3rd, Bob and Squeeze were *Geochelone sulcata*; Leo male and Leo female *Stigmochelys pardalis*.

Number of Sessions	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Dawn	3	3	1	4	3	3	4	3	4	2	4	3	4	1	4	4	3
3rd	4	3	4	4	5	5	4	4	4	4	3	4	2	5	2	1	3
Bob	2	2	4	5	5	4	3	3	3	4	5	6					
Squeeze	3	4	3	4	3	5	2	3	0	2	5	4	4	4	5	5	
Leo male	2	3	6	3	6	5	5	4	5	2	2	5	3	4	4	3	4
Leo female	0	2	3	2	2	1	4	4	3	3	5	2	5	2	3	3	4

Scent Test

The two tortoises that reached the learning criterion then received 12 trials of the Scent test. Bob made eight correct choices whilst Squeeze made nine. A Wilcoxon test comparing the test results to the training trials of each individual separately, in which the learning criterion was reached, revealed that there was no significant difference in the performance of the subjects ($p>0.05$).

Feature – Test

Bob was run on the Feature Test after he had completed the Smell Test. Figure 6 shows the number of times that Bob chose colour compared to shape. A Binomial test revealed that this difference was not significant ($p>0.05$). Squeeze was not tested in the feature test as bad weather conditions prevented testing.

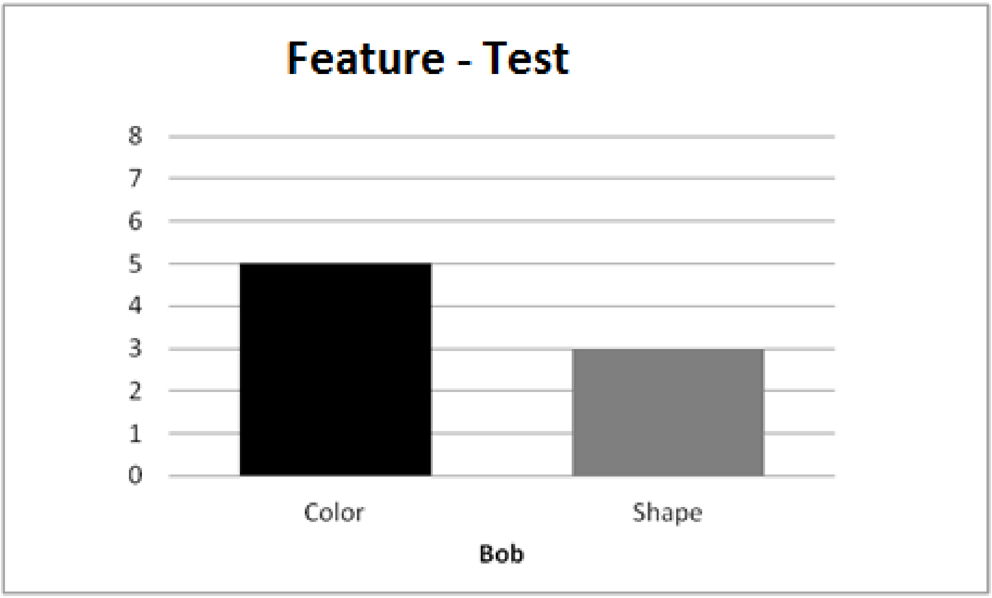

Figure 5: Number of choices according to color (left) and shape (right) made by Bob during the Feature test.

DISCUSSION

This study shows for the first time that tortoises are able to learn to associate different visual cues with distal target locations in a T-maze. Further, they did not solve the task using odour cues but rather used some element of the stimulus to solve the task. The results of the Feature test suggest that both colour and shape of the cue could play a role in the learning process.

Two different stimuli, varying in both colour and shape were used as cues for distal food being present at different positions in the maze. Most previous discrimination studies tested two colours or shapes, black and white, or different shades of grey against each other (for a review see Burghardt, 1977). The additional task by combining each cue with a specific direction used in a T-maze means not only discrimination learning of cues, but also associates each cue with one side of the maze. This task is a combination of discrimination learning and orientation and that might increases the level of difficulty in it. Two of the six tortoises reached the learning criterion and were able to solve the task, however, the other four failed.

After the actual training the two individuals that reached the criterion were tested in the Scent test to find out if odour cues of food were involved. Both of them showed no decrease in their performance when compared to training trials. Therefore, this suggests that they did not use any odour cues to navigate in the maze but rather orientated using the visual cues that were presented to them. There is still no experimental evidence why tortoises in general do not use smell in experiments. It seems that the visual sense is more important to them as the same results also occurred in earlier studies by Wilkinson et al. (2007, 2009) with the red-footed tortoise using different set-ups. As mentioned before, experiments of Davis and Burghardt (2007) revealed the same phenomenon in their visual discrimination task with Florida red-bellied cooters. They ran additional odour control tests and found out that the turtles only used visual cues. In Wilkinson, Mueller–Paul and Huber (2012) it is suggested that after findings of Hansknecht and Burghardt (2010) it is possible that tortoises learn to ignore odour cues during the training period and focused on the visual stimuli. It supports the idea that the visual sense is more important, not only in the red-footed tortoise (Wilkinson, Mueller-Paul and Huber, 2012) but maybe for chelonians in general (Davis and Burghardt, 2007).

The results of the Feature test revealed a disruption in performance suggesting that Bob used both features of the cue to make his decision. Further testing with more individuals and test trials could reinforce these findings. One individual is a very small sample size, but may nevertheless be, to some extent, indicative of the capabilities of the species. As it is difficult to generalize from one stimulus set to another, different sets of stimuli should be considered in further testing.

Interestingly, the pilot study revealed that the tortoises were not motivated to work for a shelter. This aspect was included in the study because of the ecological conditions (Gurley, 2002) in their natural habitat in Africa. Shelter could have been rewarding for them as tortoises in general like to hide in shelters, burrows or bushes (Gurley, 2002; Loveridge and Williams, 1957), especially sulcata tortoises that usually dig their own burrows (Gurley, 2002). However, both species showed no interest. It is known that spurred tortoises do not dig as much in captivity as in the wild, if they have everything they need (Gurley, 2002). That could be a possible explanation why shelter was not rewarding for them. Food was motivating enough throughout the entire study.

The comparative approach of using leopard and sulcata tortoises derived from their different ecological strategies in terms of shelter use and food preferences, but living in similar habitats (Gurley, 2002; Loveridge and Williams, 1957). These differences could have influenced their behaviour or abilities in this task. No leopard tortoise completed the task whilst two sulcata tortoises reached the learning criterion, but because of the small sample size the data of the present study are inconclusive. Nevertheless, comparative studies are very important. Further studies with different breeds which differ in ecology, habitat and with bigger sample sizes may improve our knowledge about the capabilities of tortoises. Habitat and ecological needs could influence memory and learning behaviour and therefore need to be taken in account. Newman (1906) was a pioneer in studies of comparative intelligence in turtles. He observed different species in their natural habitat as well as in captivity and already noticed differences in their behaviour. Therefore he claimed that studies on captive animals could be misleading and emphasized the importance of studies on different breeds (Burghardt, 1977). An important topic discussed in reptile papers (Jacobs, 2003;

López, Gómez, Rodríguez, Broglio, Vargas and Salas, 2001) and also in the current study is the phylogenetic relationship between chelonians, archosaurs (birds, crocodiles) and lepidosaurs (lizards, snakes). More comparative cognitive studies within these groups could lead to a better understanding of their phylogenetic relationships. In particular, studies of their different brain structures (O'Keefe and Nadel, 1978; Macphail, 1982) as well as learning and discrimination studies in reptiles (Burghardt, 1977) and studies that consider their close relationship to birds (López, Gómez, Rodríguez, Broglio, Vargas and Salas, 2001; Cao, Sorenson, Kumazawa, Mindell, and Hasegawa, 2000) would be required.

This study has shown that tortoises indeed have the capability to discriminate two cues that differ in colour or shape after a proper training phase and to use them to find a distal goal. Previous studies already showed that tortoises are able to discriminate colours as well as shapes (Casteel, 1911; Kuroda, 1933; Wojtusiak, 1933, 1934), and this study confirmed the importance of the visual sense in tortoises (Burghardt, 1977; Gans and Ulinski, 1992) also in terms of orientation. Now that the experimental set-up is worked out for tortoises it would be quite interesting to carry out further comparative studies with them. The present study is just a beginning; more data collection in feature testing may confirm the current knowledge and extend it. Another interesting following up study would be retesting after a few weeks or months the tortoises that learned the task, to improve our knowledge about long term memory in this species.

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Appendix

Zusammenfassung

Forschungsergebnisse mit Schildkröten haben gezeigt, dass sie in der Lage sind, sich anhand distaler Markierungen räumlich zu orientieren. Des Weiteren konnte gezeigt werden, dass sie eine kognitive Representation der Umgebung sowohl zum Navigieren als auch zur Unterscheidung verschiedener Farben und Formen verwenden können. In der vorliegenden Studie wurde untersucht, ob es für sie auch möglich ist, lokale Markierungspunkte in ihrer Umgebung zu unterscheiden und sie zu nutzen, um an ein distales Ziel zu gelangen. Dieser Prozess verlangt vor allem die Fähigkeit, verschiedene Zeichen, so genannte "Cues", zu unterscheiden und sie mit einer bestimmten Richtung zu assoziieren. Dazu wurden Sporn - *(Geochelone sulcata)* und Pantherschildkröten *(Stigmochelys pardalis)* darauf trainiert, sich anhand zweier Zeichen, die sich in Farbe und Form unterschieden, in einem T-förmigen Labyrinth zurechtzufinden. Die zwei Zeichen wurden mittig gegenüber der Startposition angebracht. Eines der Zeichen bedeutete eine Belohnung im rechten Arm des Versuchsaufbaus, das andere Zeichen bedeutete eine Belohnung auf der linken Seite. Die Ergebnisse zeigten, dass Spornschildkröten in der Lage waren, dies zu lernen. Danach wurden die Tiere darauf getestet, ob auch Gerüche eine Rolle in der Orientierung spielten. Wie erwartet stellte sich heraus, dass dies nicht der Fall war. Im Anschluss daran wurde getestet, ob Farbe oder Form der „Cues“ wichtiger in dem Lernprozess waren. Die Leistung in diesem Test gab einen Hinweis darauf, dass Farbe, Form oder beides zusammen wichtig für die Diskrimination sein könnten. Dies bedeutet, dass Schildkröten mit ihrer visuellen Wahrnehmung verschiedene Farben und Formen zu unterscheiden vermögen, und dass sie in der Lage sind, deren Funktionen mit Richtungen zu verbinden. Der Vergleich der beiden Arten, die ähnliche Lebensräume bewohnen, sich aber in ihrer Ökologie unterscheiden, zeigte auf Grund der geringen Datenmenge keine signifikanten Ergebnisse.

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