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DISSERTATION

Titel der Dissertation

„Spatial cognition in reptiles:

Short-range navigation in the red-footed tortoise and the jewelled lizard“

Verfasserin

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Author contributions

Chapter 1: Introduction

I wrote the introduction.

Chapter 2: Spatial Cognition in reptiles

I did the literature search and wrote the chapter. The co-authors were involved in editing paper.

Chapter 3: Radial-arm maze navigation of the red-footed tortoises (*Geochelone carbonaria*)

I designed this study with input from my co-authors. I collected and analysed the data and wrote the paper. The co-authors were involved in editing the paper.

Chapter 4: Response-stereotypy in the jewelled lizard (*Timon lepidus*) in a radial-arm maze

I designed this study with input from my co-authors. I collected and analysed the data and wrote the paper. The co-authors were involved in editing the paper.

Chapter 5: **Social learning in a non-social reptile (*Geochelone carbonaria*)**

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Chapter 6: **Picture-object recognition in the tortoise *Chelonoidis carbonaria***

I designed this study together with Anna Wilkinson. I collected the data, did some data analysis and wrote parts of the paper.

Chapter 7: **Red-footed tortoises (*Geochelone carbonaria*) are capable of transferral between the touch screen and a physical arena**

I designed this study together with my co-authors. I collected and analysed the data and wrote the paper. Anna Wilkinson and Ludwig Huber were involved in editing the paper.

Chapter 8: **Conclusions**

I wrote the conclusions.

Summary

Animal cognition is the study of cognitive processes such as learning and memory in a variety of species. While the cognition of mammals and birds has been researched intensely, not much is known about reptile cognition. However, because of the evolutionary connection between the orders a sound knowledge of reptile cognition is needed to fully understand the evolution of cognition. It was the aim of the present thesis to contribute to the better understanding of reptile spatial cognition.

After an overview on cognition research and reptile spatial navigation provided by the first two chapters, chapter 3 and 4 describe studies on eight-arm radial maze behaviour in the red-footed tortoise (*Geochelone carbonaria*) and the jewelled lizard (*Lacerta lepida*), respectively. Both the tortoises and the lizard showed a strong preference for using response stereotypic behaviour when navigating the maze. Thus, compared to mammalian navigation mechanisms, reptile spatial behaviour might rely more strongly on intrinsic cues. Chapter 5 describes a study showing that red-footed tortoises were able to master a spatial detour task after observing a conspecific demonstrator while they were unable to manage it by themselves. Given the solitary nature of this species the ability to learn socially suggests access to a large problem-solving repertoire. The study described in chapter 6 examined the ability of red-footed tortoises to perceive and comprehend 2D images. The findings showed that they were able to distinguish food and non-food items when presented with 2D photographs. Chapter 7 presents findings showing the ability of red-footed tortoises to use a touchscreen, a skill hitherto undemonstrated in reptiles. Furthermore, the tortoises were able to transfer knowledge acquired in the touchscreen set-up to a ‘real-world’ 3D arena.

In the final General Discussion I have summarized the most important data of my five empirical studies and conclude with the question of what this tells us about reptilian cognition. The limited amount of data suggest that cognitive processing of mammals, birds,

and reptiles are quite similar. However, to enable direct comparisons of the different orders and to achieve a conclusive level of evidence, much more basic research is needed.

Zusammenfassung

Tierkognition ist ein Forschungsfeld, dass sich mit Lern- und Gedächtnisprozessen von einer Vielfalt von Arten beschäftigt. Obwohl der Bereich der Säuger- und Vogelkognition recht gut erforscht ist, weiß man bis heute nur wenig über die Kognition von Reptilien. Aufgrund der der evolutionären Verbindung zwischen den Klassen ist ein detailliertes Wissen über Reptilienkognition jedoch notwendig um die Evolution der Kognition vollständig verstehen zu können. Es war das Ziel dieser Dissertation zum besseren Verständnis der räumlichen Kognition von Reptilien beizutragen.

Nach einen Überblick über Kognitionsforschung und räumlicher Orientierung von Reptilien in den ersten beiden Kapiteln, beschreiben Kapitel 3 und 4 Studien über das Verhalten von Köhlerschildkröten (*Geochelone carbonaria*) und Perleidechsen (*Lacerta lepida*) im Sternlabyrinth. Beide Arten zeigten eine starke Präferenz für Bewegungslernen, was darauf hinweist, dass sich Reptilien im Vergleich zu Säugern und Vögel mehr auf intrinsische Hinweise verlassen könnten. Kapitel 5 beschreibt eine Studie die zeigt, dass Köhlerschildkröten in der Lage waren den Weg um einen Zaun zum Futter zu finden wenn dies zuvor von einen Artgenossen demonstriert wurde, nicht aber ohne die Demonstration. Da Köhlerschildkröten Einzelgänger sind weist dies darauf hin, dass sie ein großes Problemlösungsrepertoire haben. Die in Kapitel 6 beschriebene Studie zeigt, dass Köhlerschildkröten Futter und Nicht-Futter Gegenstände auf Fotos unterscheiden können und somit in der Lage sind zweidimensionale Bilder zu erkennen und zu verstehen. Kapitel 7 präsentiert Ergebnisse, die die Fähigkeit von Köhlerschildkröten einen Sensorbildschirm zu bedienen belegen, eine Begabung die bislang in Reptilen noch nicht gezeigt wurde. Des Weiteren waren die Schildkröten in der Lage Wissen welches sie am Bildschirm erlernt haben in eine dreidimensionale Arena zu übertragen.

In dem abschließenden Diskussionskapitel habe ich die wichtigsten Daten meiner fünf empirischen Studien zusammengefasst und schließe mit der Frage was diese für die Kognition von Reptilien bedeuten. Basierend auf der jedoch deutlichen begrenzten Datenmenge, die in Bezug auf Reptilienkognition existiert, ist anzunehmen, dass die Kognitionsprozesse von Reptilien denen von Säugern und Vögeln größtenteils ähneln. Bevor allerdings direkte Vergleiche zwischen den Klassen und beweiskräftige Schlussfolgerungen gezogen werden können muss noch viel Basisforschung betrieben werden.

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Chapter 1: Introduction

Julia Paul

Introduction

Animal cognition is concerned with the study of cognitive processes such as learning, memory and thought in a large variety of species (Emery & Clayton, 2008). Given the vast number of topics that can be learnt, memorised and generally processed cognitively, the field is divided into a number of subfields such as visual, social, and spatial cognition to mention just a few. Spatial cognition, the main area of research discussed in this thesis, is concerned with the processing of spatial information with respect to an animal's environment and to the animal itself. Processing of spatial information is essential for successful orientation and navigation, which in turn is essential for survival. However, the various areas of animal cognition are by no means unconnected. Thus, the present work will provide links to the areas of social cognition, showing how social interactions can influence spatial choices, and visual cognition, as a prerequisite for testing certain aspects of spatial cognition.

Cognition is an important survival function of many different species. However, due to test subject availability and, presumably, ease of testing, some groups have received far more attention than others. As such, mammal and bird cognition has been studied intensely for many decades, while reptile cognition has been largely neglected. Such a development is likely to have resulted, in part, from the specific care that needs to be taken when testing reptilian subjects. Firstly, due to their ectothermal nature, it is essential to create a temperature environment appropriate to the study species, not only in the home enclosure but also during testing. Furthermore, many reptile species are much less domesticated than many mammals or birds, and particularly compared to the commonly used small rodents. As such, the somewhat lengthy period of taming and familiarisation not only with the test apparatus but also with human handling is often needed before the animals are able to demonstrate their full cognitive potential in laboratory tests. As the field of reptile cognition is still comparatively unexplored the present study took particular care to avoid anthropomorphism or anthropocentrism and to abide by Morgan's canon.

The present chapter will firstly provide an overview of the scientific field of spatial cognition and then secondly, introduce some of the challenges of studying reptiles and their cognition. It will further give a brief description of the ecology of the two study species tested in the course of this work: the red-footed tortoise (*Geochelone carbonaria*) and the jewelled lizard (*Lacerta lepida*). The last section of this chapter will outline the aims and structure of this thesis.

Spatial cognition

Spatial cognition refers to the learning and remembering of spatial information which give an animal a clear survival advantage when travelling between resting places, feeding grounds and nesting locations (Emery & Clayton, 2008). The brain region believed to be most heavily involved in spatial processing in mammals is the hippocampus (O'Keefe & Nadel, 1978) and in birds the medial telencephalon, also referred to as the avian hippocampus (Macphail, 2002). Reptiles possess neither a hippocampus nor a medial telencephalon. The region in the reptilian brain, which has been suggested to be a homologue of the hippocampus, is the medial cortex (Macphail, 1982). However, only little evidence to date has tested the degree of analogy in behaviour patterns. While most behavioural evidence suggests that reptilian spatial behaviour is comparable with that of mammals and birds (López et al., 2000, 2001, 2003, Wilkinson et al., 2007), some evidence from lizards suggest differences in behavioural strategies (Day et al., 1999, 2001). Thus, more carefully designed research into the strategies used by different reptile species is needed.

Cues from different environmental domains can be used to orient in space, with some kinds of cues supporting long-distance navigation, while others enable short-range travel within the more familiar home range of an animal. Cues such as geomagnetic maps serve far ranging migrations as seen in some birds and sea turtles (Lohmann, Lohmann & Putman, 2007), while celestial cues such as sky polarisation patterns have also been shown to support animal long-distance travel through unfamiliar territory (Alerstam, 2006). Cues that are useful and necessary for long-distance travel, however, often do not prove successful at short distances.

When an animal stays in one comparatively small area for some time, whether this is after reaching the end of its migration or because that area becomes its permanent home range, other navigation strategies appropriate for distinguishing places on a very small scale are required. Such short-distance navigation has been studied intensively in the lab, particularly in rodents. Short distance navigation strategies can be either extrinsic or intrinsic (Roberts, Cruz, & Tremblay, 2007). Extrinsic cues are produced by the environment, and are, for example, visual or olfactory in nature. Intrinsic cues are generated by the animal itself and are based on its movement through space.

While chemically based olfactory cues exist and may help orientation in many environmental settings, visual cues seem to be prevalent in most navigational strategies (Emery & Clayton, 2008). In respect to visual spatial orientation two main strategies have been identified: beacon learning and formation of cognitive maps (O’Keefe & Nadel, 1978; Tolman, 1948). A beacon is a distinct, obvious landmark, which is positioned close to a goal. As such, it is a very simple indicator of a goal and moving towards it on the most direct route promises success. One drawback of navigation based on beacons is that an appropriate beacon near the desired goal has to be present in first place. Another disadvantage is that orientation breaks down entirely when the beacon is removed.

Navigation relying on the formation of cognitive maps successfully tackles these problems. Cognitive maps are mental representations of one’s environment that incorporate all, or many, of the surrounding cues. In this case no one particular landmark needs to be close to the goal and no one landmark is essential. This implies that reaching the goal is still possible when some of the visual cues disappear or are spontaneously blocked from sight. As such, while posing higher demands on memory, cognitive map formation is more reliable and flexible in an ever-changing natural environment. However, hierarchically, cognitive map formation appears to be overshadowed by beacon learning. López et al (2001), for example, showed that turtles (*Pseudemys scripta*) were able to learn to navigate towards a goal using a cognitive map-like strategy, but chose to ignore these distal cues when a beacon was present during training.

While much evidence shows the existence of visually guided navigation, neither visual, nor olfactory, nor any other extrinsic cues, however, are indispensable for successful navigation around a radial-arm maze or towards a specific goal. Given stable external conditions, as are often generated in laboratory experiments, orientation is also possible based entirely on intrinsic cues. One possible internal cue is 'dead reckoning', which is used interchangeably with the term 'path integration'. Dead reckoning refers to vestibular, idiothetic feedback generated by one's own body that provides information about the distance and direction travelled from a starting point (Shettleworth & Sutton, 2005). It is indicated by an animal's ability to return from a goal to a starting point on a direct route after a goal had been approach via several detours. Hierarchically, dead reckoning appears to be comparatively independent and does not seem to interfere to a great extent with visual cue learning (Shettleworth & Sutton, 2005). However, navigations seems to be much more accurate when both types of information are present and animals tend to rely more on visual cues when these are available (Etienne, Maurer & Séguinot, 1996).

Another strategy that does not require external cues is 'response stereotypy'. This refers to the learning of response patterns, such as executing a fixed series of left and right turns. Pigeons (*Columba livia*), for example, learn to take a certain route in an open field search task to get a goal position when they are not provided with visual cues (Sutton & Shettleworth, 2005). Similarly, a red-footed tortoise used a specific sequence of identical turns to solve a radial-arm maze when no visual cues were provided (Wilkinson et al., 2009). This navigational strategy is very reliable and easy to acquire, as long as the environment is entirely stable. However, as soon conditions change the previously successful rule might not apply anymore. Sutton and Shettleworth’s (2005) pigeon’s, for example, were only successful at solving their search task from a novel entrance position when they had previously been trained with access to visual cues. Those pigeons that had successfully solved the task without visual cues on the basis of response stereotypy failed this test. Thus, the disadvantage of travel based on intrinsic cues is, similar to beacon-based navigation, its lack of flexibility. When circumstances change a new set of response rules can be formed, which however requires a lengthy trial and error search phase every time the environment changes.

When comparing navigational strategies it appears that cognitive map formation, a strategy that is comparatively complex to learn and that creates a high memory load, is more flexible and versatile than those that are easy to learn, such as response stereotypy and beacon learning. Therefore, strategies are often combined, making use of the flexibility of cognitive maps and relieving memory load by applying, for example, an additional response stereotypic rule. This behaviour has been demonstrated by rats in a radial-arm maze task (Bond, Cook, & Lamb, 1981; Dale, 1981). The rats applied a simple response strategy but tests showed that these strategies were secondary to visual memory strategies and that the rats did not ignore the visual cues.

One very commonly used method of testing spatial navigation strategies and cognitive map formation in animals is the radial-arm maze. This has been developed by Olton and Samuelson (1976) for use with rats and has since gained great popularity for testing spatial navigation in a variety of species. It consists of a central platform from which a certain number, usually eight, equally spaced arms are radiating. At the end of each arm there is a hidden food reward, which the animal will only discover after making a clear choice by walking into an arm. The food reward is generally eaten during the first visit to an arm and will not be replaced during a trial. Thus, if an arm is revisited, no reward will be found. Efficient performance requires memory of already visited arms to avoid unnecessary wanderings into arms that have already been depleted. Successful radial maze use has not only been demonstrated in rats (Bond, Cook, & Lamb, 1981; Cook, Brown, & Riley, 1985; Dale, 1986; Dale & Innis, 1986; Olton & Collinson, 1979; Olton, Collinson, & Werz, 1977; Olton & Samuelson, 1976; Roberts, Cruz, & Tremblay, 2007; Roberts, & Dale, 1981; Singer, Abroms, & Zentall, 2006) but also in gerbils (Wilkie & Slobin, 1988), humans (Aadland, Beatty, & Maki, 1984), pigeons (Bond et al., 1981; Gagliardo, Mazzotto, & Divac, 1997; Olson & Maki, 1983; Roberts & Veldhuizen, 1985; Zentall, Steirn, & Jackson-Smith, 1990), different corvid species (Balda & Kamil, 1988; Kamil, Balda, & Olson, 1994; Olson, Kamil, & Balda, 1992), fish (Hughes & Blight, 1999; Roiblat, Tham, & Golub, 1982), and tortoises (Wilkinson et al., 2007; 2009).

The radial-arm maze, by design, tests the subject’s ability to remember several locations and their visiting history within a single trial. This type of design is referred to as win-shift and is contrasted to win-stay paradigms. Win-stay tasks require the animal to learn and remember one specific location and to return to this location once during every trial. As such, while both design types require long-term memory, win-shift tasks put additional demands on working and short-term memory. Both paradigms have ecological relevance. For foraging in different locations it is useful to remember where one has already been and which food sources have already been depleted. However, some food sources might be very extensive and stand alone as a daily goal. Also, nesting areas and sleeping quarters are stand-alone goals which an animal needs to be able to find with ease. The present thesis will give examples of tests of both paradigms and show that red-footed tortoises are capable of succeeding on tests of either paradigm. The thesis will also give an idea of the great importance of non-visual, intrinsic cues when orienting in a radial-arm maze.

Studying reptiles and their cognition

Reptiles have common ancestral roots with birds and mammals. There is some debate about exactly when, and in which order, these groups have split from one another (Zardoya & Meyer, 2001), however, approximations indicate that they have shared a common ancestor around 225 million years ago (MacPhail, 1982). While the existence of a common heritage forms the basis for common traits nowadays, the vast time span since the split provides immense possibilities for divergence of the evolutionary paths of the amniotic groups. Some behavioural studies have indicated clear similarities between the cognitive strategies used by reptiles and mammals (López et al., 2000; Wilkinson, Chan, & Hall, 2007), but others have found behavioural differences (Day, Crews, & Wilczynski, 1999).

Reptilian brain structures have been shown to be substantially different from those of mammals, particularly when comparing the thin-layered forebrain region of reptiles with the multi-layered one of mammals (MacPhail, 1982). Given both these similarities and differences, a structured comparison of the cognitive skills and strategies used by reptiles with those of mammals and birds is called for. In fact, for a well-rounded understanding of the evolution of cognition it is essential.

However, in comparison to the body of research concerned with mammal and bird cognition very little work has examined reptiles (for a review see Wilkinson & Huber, in press). This is not due to a late recognition of reptiles as experimental subjects. Yerkes (1901) studied the formation of habits in the turtle at the very beginning of the twentieth century. However, early attitudes towards reptiles and their abilities might have discouraged later researchers from working with them and have thus led to the relative neglect of the area: “Reptiles are usually considered sluggish and unintelligent creatures, and there can be no question about the general truth of this opinion. Turtles certainly appear to be very stupid – so much so, indeed, that one would not expect much in the way of intelligent actions.” (Yerkes, 1901, p. 520). The present thesis is one of several current attempts to challenge this attitude towards reptiles in general and chelonians (turtles, terrapins, and tortoises) in particular. It is aimed at providing a small step towards a better understanding of reptilian abilities and of their cognition in tasks which have traditionally been used with mammals and birds. However, because of the small amount of basic information on reptilian behaviour studies that are truly comparable to those run with mammals and birds, will only be possible once much groundwork is done and many obstacles are tackled.

The study of reptiles is to some respect restricted, or at least, guided by certain environmental conditions and constraints due to their ectothermal nature. This might be one reason why, to date, there is so little cognitive research on reptiles compared to the far more exhaustive base of knowledge on mammals and birds. As reptiles rely on external sources of energy for their thermoregulation it is necessary to provide ideal, species-specific environments (Huey, 1982), when attempting to study them. As such, maladjusted temperatures in a test room can have a number of effects from endangering the reptiles' health by overheating through slowing down the animal's reactions to entirely disabling the reptile's ability to move. Consequently, caution has to be taken to maintain stable test room temperatures across training and test sessions to avoid introducing artificial biases based on the reptile's thermal ecology.

Due to their low metabolic rate, reptiles require far less resources in terms of food and water compared to mammals and birds (Huey, 1982). While this is a clear survival advantage in the wild, it creates further need for consideration with respect to the planning and running of laboratory experiments with reptiles. Experimental designs providing food rewards need to be carefully adjusted to the animals' appetite, which poses a restraint on the number of trials that can be run per day. As such care needs to be taken when comparing learning curves of reptiles, mammals, and birds in analogous experiments, as the time span over which learning of the same number of trials has taken place might vary greatly.

In conclusion, the study of reptile cognition has the potential to be highly informative about the evolution of cognition, but much research is needed to fill the gap in the pool of knowledge on cognitive processes. Furthermore, studying reptiles is not without challenges and requires high awareness of the subjects' ecology, intense experimental planning, and, not least, a great amount of patience with a subject that is, despite its long-standing tradition, still in its infancy. An attempt to tackle these challenges was made throughout the production of the present thesis with the goal of producing a contribution towards increasing the knowledge on reptile spatial cognition. The next sections will discuss the ecology of the chosen study species, red-footed tortoises (*Geochelone carbonaria*) and jewelled lizards (*Lacerta lepida*), and explain the reasons for choosing these particular species.

Ecology of the red-footed tortoise

Red-footed tortoises (*Geochelone carbonaria*) belong to the order Testudines, suborder Cryptodira, family Testudinidae and the genus Chelonoidis. They reach a carapace size of 30 to 50 cm with males reaching a slightly larger size than females (Moskovits, 1988). Further signs of sexual dimorphism are variations in shell shape (*Figure 1*), with males showing a clearly concave plastron and a prominently incurved carapace. Females sport a rather evenly shaped carapace and a relatively flat plastron. However, the strength of expression of this dimorphism seems to vary across regions. While a clear distinction between the sexes can be made on purely visual grounds in Brazil (Moskovits, 1988), a large reference sample is required to clearly determine the sex of red-footed tortoises from Panama by the shape of their shells (Legler, 1963). In addition, Legler (1963) found that males have longer, differently shaped tails compared to females. Red-footed tortoises reach maturity at approximate sizes of 20 to 25 cm. Juvenile and sub-adult tortoises have an evenly domed, straight-sided carapace and a flat or slightly convex plastron (*figure 1*, Legler, 1963). With age and increase in size the relative height of the animals is reduced.

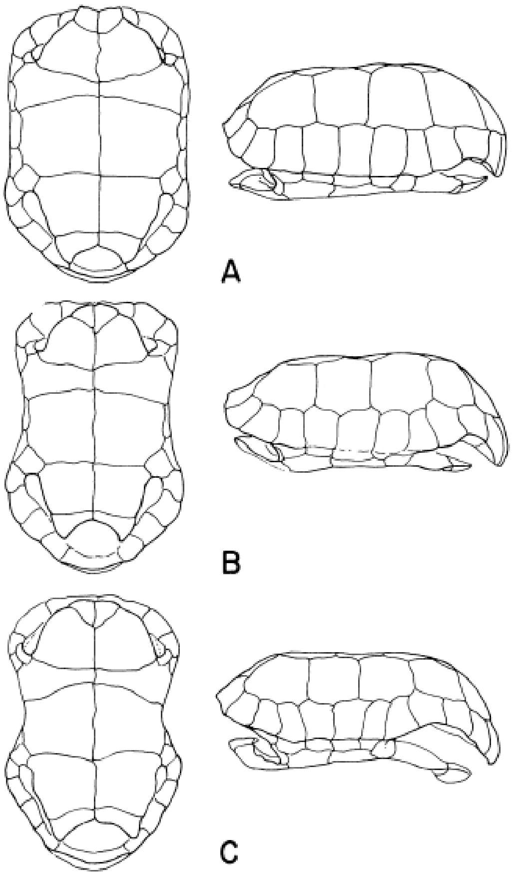

Figure 1: Shape examples of a (A) juvenile, (B) adult female, and (C) adult male red-footed tortoise (reproduced from Legler, 1963).

During the breeding season male red-footed tortoises have been observed to challenge every object that approximately matches themselves in size with a jerky, horizontal head-having motion (Auffenberg, 1965). This movement is only reciprocated in the identical manner by males of the same species. Thus, recognition of mature males of the same species is achieved with a single simple action. Recognition of adult females usually requires two steps. Initially, the female is approached with the aforementioned head movement. If the male receives no reaction to this he walks around to sniff the cloacae of the other tortoise. This examination tells the male whether the female is able to receive and leads to the initiation of courtship, during which vocalisations are common (Auffenberg, 1965). Vocalisations of this species have not only been observed in association with mating behaviour, where a series of cluck-sounds is produced but are also produced by juveniles, who emit a single cluck-sound when moving about (Campbell & Evans, 1967, 1972).

Autopsy data showing eggs at very different developmental stages indicate that red-footed tortoises lay several clutches of eggs per year (Vanzolini, 1999). Clutch sizes have been reported to lie between one and 15 elliptical-shaped eggs. The reproductive state of the females indicates full reproductiveness approximately two months before the peak of the rainy season (Vanzolini, 1999).

Red-footed tortoises are endemic to vast areas of Central and South America from Venezuela to Northern Argentina (Bruins, 2006). Their preferred habitats range from moist savannas to the edges of rain forests (Moskovits, 1988) where they require day time temperatures between 25 and 30 °C, night-time temperatures of 20 to 22 °C and a basking temperatures of up to 45 °C. They naturally appear in areas with humidity of around 80%. Red-footed tortoises are active during the day. They spend much of their time sheltering in existing burrows and under naturally occurring covers, but do not create shelters themselves (Bruins, 2006).

The diet of red-footed tortoises consists primarily but not entirely of plant matter. Fruit make up the largest part of their diet, followed by flowers (Pingleton, 2001). They generally accept a very large range of different fruit and flowers but do demonstrate individual preferences for certain foods (Moskovits & Bjorndal, 1990). When encountered, protein-rich animal matter is also consumed and can be considered a favourite. In addition they have been observed to feed on live and dead foliage, stems, fungi, soil, and sand. While generally solitary the tortoises often come together in small groups under fruiting trees (Moskovits & Bjorndal, 1990).

Red-footed tortoises have been shown to be effective seed dispersal agents for a number of different plants such as *Ficus sp.* and *Aechmea sp.* (Strong, 2006). Their minimum dispersal rate was estimated at 91 m, with the likelihood that seeds are usually dispersed much further than this. Strong (2006) recorded that the mean distance travelled daily was 57 m, with some days of complete rest and other days showing travel distances of up to 400 m. It appears that the tortoises have a tendency to remain within certain areas for, at least, some time, as relatively high re-capture rates show (Moskovits, 1988). Overall, red-footed tortoises spend large proportions of time resting, being inactive over 50% of their time. Male red-footed tortoises are at their most active during the breeding season possibly searching for the largest possible number of mature females to mate with (Moskovits & Kiester, 1987). In comparison, female red-footed tortoises are less active during mating season and more active during nesting season, when they are required to search for appropriate nesting locations.

The relatively large distances red-footed tortoises travel while looking for food sources, mating partners, and nesting locations suggests that they require the ability to orient themselves well in their environment. This combined with the relatively high recapture rate and the fact that groups of tortoises gather repeatedly under fruiting trees might be an indication that they have the potential for spatial learning and knowledge to guide them to specific locations on their sometimes extensive travels. As such, red-footed tortoises are likely to be a useful species for studying spatial cognition.

Ecology of the jewelled lizard

Jewelled lizards (*Timon lepidus*) are a ground living species of wall lizard belonging to the order Squamata, suborder Sauria, family Lacertidae, and genus Timon. They are the largest European lacertid lizard with a head and body length of up to 20 cm and a total length of 60 to 80 cm for males and 50 cm for females. A further sexual dimorphism is body shape, with females always displaying larger abdomen, and males larger heads. The abdomen-head ratio of juveniles of both sexes combined is comparable to that of males but to lower than that of females (Braña, 1996).

Jewelled lizards are endemic to the dry, shrubby, thickly vegetated hills of the Mediterranean (Bruins, 2006). Microhabitat studies have placed them close to rocks with an abundance of sizable crevices. The availability and easy accessibility of retreats as predators approach seems to be the main determinant for microhabitat choice of the lizards (Días, Monasterio, & Salvador, 2006). Given the availability of such retreats they are found in both cultivated and uncultivated areas. However, at least in some cases, the presence of humans has led to reduction in the number of jewelled lizards. Particular dangers of human proximity are not only the human activities such as picnics in the lizards' habitat themselves but also feral and semi-feral cats and dogs which are often associated with human settlements and have been observed to feed on large lizards (Allen, 1977).

Jewelled lizards are mainly active during the day between April and October and hibernate in winter. Hibernation, however, is not associated with significant weight loss which indicates that energy requirements during the winter months are very small (Castilla & Bauwens, 1990). During the summer months they require day-time temperatures between 23 and 28 °C, night time temperatures of 16 to20 °C, and basking temperatures of 35 to 45 °C. In captivity they should be provided with humidity levels of around 60% (Janitzki, 2008). Male jewelled lizards are territorial, particularly during the mating season in spring (Castilla & Bauwens, 1990). During this time they spend great amounts of energy on activities related to reproduction, such as acquisition and defence of territories, searching for and guarding mates, and courtship and mating. Females usually oviposit in July and, due to the obvious climate restraints, reproduction is generally fairly synchronised in this species (Castilla & Bauwens, 1990).

The diet of jewelled lizards consists largely of invertebrates of various sizes. Coleoptera or beetles have been found to be the most consumed group, but a range of other invertebrates such as arthropods and snails were consumed too (Castilla, Bauwens, & Llorente, 1991; Hódar, Campos, & Rosales, 1996). Within these groups however, high selectivity and clear prey selection has been found (Hódar et al., 1996). Of much lesser importance to their diet appear to be small vertebrates which were only occasionally encountered. Castilla et al. (1991) found that fruit had been consumed by 80% of the examined lizards, however, the amount of fruit was very small. When fruit was consumed this was usually the Caper and other fruit were nearly only consumed when the Caper was not available (Hódar et al., 1996). The consumption of some fruit might be due to jewelled lizards having a body mass at the border of the shift from insectivorous to herbivorous as described by Plough (1973). No differences seem to exist in the diet of males and females but a larger range of prey in larger animals, presumably due to prey size restrictions of the smaller animals, has been reported (Castilla et al., 1991).

Hódar et al. (1996) report that jewelled lizards feed on two nocturnal beetle species. As the lizards are said to be diurnal they are likely to search for the beetles during the day. They can then either dig for the beetles immediately or wait and extend their hunting periods into twilight to overlap with the active periods of the beetles. Either method, however, suggests active foraging on the part of the lizard without direct sighting of prey movements. Such prey search behaviour is unusual in lizards and is very advantageous for the design of spatial search paradigms in the laboratory (Hódar et al., 1996). Thus, their active prey searching behaviour without the need for visually perceiving prey movements in combination with their relatively large size and high energy requirements suggests that jewelled lizards are a useful study species for spatial cognition experiments that reward subjects with food and require active foraging. An example of such a task is the aforementioned radial-arm-maze.

Aims and structure of this thesis

The focus of this thesis is to investigate several interrelated elements of spatial cognition of reptiles. Chapter 2 provides an overview of three different areas of spatial cognition: long-range navigation and the geomagnetic map, homing and the celestial compass, and short-range navigation. The remainder of this thesis can be placed within the section of short-range navigation, which mainly describes highly controlled laboratory experiments on a range of different reptiles. One of these experimental designs is the radial-arm maze, which was originally developed for the use in rats (Olton & Samuelson, 1976). Wilkinson et al (2007, 2009) showed that one individual red-footed tortoise was able to learn to navigate an eight-arm radial maze and that it was able to switch between two different strategies to solve the task according to room-cue availability. The first question of the present thesis was whether this behaviour was a peculiarity of this one individual and if other tortoises of the same species could also perform in a radial maze.

The study described in chapter 3 aimed to replicate and extend the findings of Wilkinson et al. (2009). The study trained and tested four red-footed tortoises on various different cue conditions. While the results confirm Wilkinson et al.'s (2009) findings that red-footed tortoises are able to solve a radial-arm maze successfully, they also suggest a different emphasis in strategy choice. The tortoises described in this chapter showed a much stronger preference for using response stereotypic behaviour when navigating the maze than previously assumed, even when multiple room cues were clearly visible. Thus, tortoise spatial behaviour might rely more strongly on intrinsic cues than that of mammals, at least under very stable laboratory conditions.

Before the next study of this thesis was run red-footed tortoises were the only reptiles that had ever been tested in a radial maze (Wilkinson et al., 2007). Thus the study described in Chapter 4 sought to extend the knowledge on reptilian radial maze behaviour by testing jewelled lizards in an apparatus and testing room identical to the one used for the tortoises in chapter 2. Jewelled lizards are only very distantly related to red-footed tortoises. They belong to the order of squamata, are mainly insectivorous and are endemic to a very different environment than red-footed tortoises. Thus, if jewelled lizards were able to perform in a radial-arm maze this would give a first indication that this ability might be general to reptiles and this is exactly what was found. The results show that the jewelled lizard is also able to solve a radial-arm maze task very quickly and efficiently. Secondly, these data suggest that jewelled lizards behave in a similar manner to red-footed tortoises in that a response stereotypic strategy is applied to solve a radial maze task.

The results described in chapter 3 showed that red-footed tortoises are able to individually learn a complex spatial task. The study presented in chapter 5 examined whether a spatial task could also be learnt socially by observation of a conspecific. A detour task was designed which enabled the tortoise to see its goal, a bowl of fruit, from the start but required it to move away from the goal around a fence to actually reach the goal. The results show that, after observing a conspecific demonstrator, red-footed tortoises were able to solve this spatial problem, which remained unsolved during individual attempts. This finding is particularly remarkable as red-footed tortoises are a solitary species. The availability of social learning in this species thus suggests that they have a large problem-solving repertoire and are able to acquire generalisable skills from a number of different sources.

To further test the generalisability of spatial learning skills in the red-footed tortoise it is necessary to test them on similar tasks in very different domains. A comparison between tasks presented on a touchscreen and in a ‘real-life’ 3D test enclosure would satisfy these domain criteria, while lending itself to designing comparable tasks. However, to sensibly use a touchscreen in the work with red-footed tortoises it is necessary to know about their visual abilities. To test whether the tortoises are able to perceive and recognise 2D images, in the study described in chapter 6 I confronted them with a picture-object recognition task. The tortoises were trained to distinguish between food and non-food items and were then presented with colour photographs of comparable items and their ability to discriminate between them was tested. The findings showed that red-footed tortoises were able to distinguish food and non-food items when presented on 2D photographs. Furthermore, the results suggest that the distinction between a real 3D food object and a photograph of a similar object is possible but very difficult for them.

Chapter 7 made use of the ability of red-footed tortoises to perceive 2D objects and presented them with a simple spatial task on a touchscreen. After reaching criterion the tortoises' ability to transfer knowledge between domains was tested by presenting them with a 3D version of the task. The tortoises spontaneously transferred their knowledge from the touchscreen to a 3D enclosure without needing further training suggesting that the knowledge they acquired is broad and generalisable. In a further stage of the experiment the tortoises were reversal trained in the 3D enclosure and then tested on the touchscreen. The results show that no transferral took place under these conditions, hinting towards an ability of red-footed tortoises to distinguish between setups and indicating the presence of long-term memory for the spatial strategy appropriate in a particular setup.

Finally, Chapter 8 concludes the present thesis summarising the overall results and embedding them in a knowledge framework. Furthermore, implications and future directions of the field of spatial short-distance navigation research in reptiles are discussed.

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Chapter 2: Spatial cognition in reptiles

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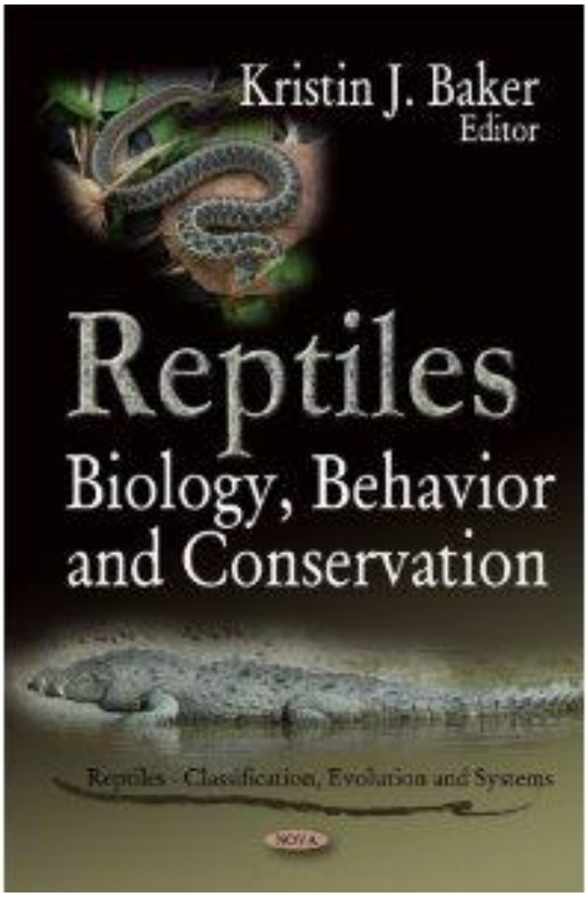

Abstract

Spatial cognition is considered an essential survival tool for almost all species as it allows an animal to navigate through complex natural environments. This skill is necessary to efficiently access food, water and shelter. While comparably little is known about the processes underlying navigational behaviour in reptiles, the cognitive processes underlying orientation in mammals and birds are relatively well understood. This chapter reviews the available literature on spatial cognition in a range of reptile species. It attempts to link research from both laboratory and field settings and interprets the evidence in terms of the behavioural ecology and evolutionary history of the specific species. Finally we discuss the brain processes which underlie spatial navigation in reptiles in light of what is known about this ability in mammals and birds.

Introduction

Work on animal cognition (on learning, memory, and information processing generally) has focused largely on the behaviour of mammals and birds. Intensive research over the last 50 years has resulted in a good understanding of their capabilities and we shall make full use of concepts derived from the study of mammals and birds in what follows. The relative neglect of reptiles in this context is to be regretted, as an analysis of their abilities has the potential to contribute substantially to our understanding of the evolution of vertebrate cognition. Mammals, birds, and reptiles have common evolutionary roots as they they and their fossil ancestors form the vertebrate group of amniotes. On these grounds it might be expected that these classes would share certain behavioural traits and capabilities. Exploration of differences and similarities between reptiles, and mammals and birds might thus provide insight about the time period in which a given cognitive trait evolved (i.e., before the amniotes split or after). On the other hand, as the split between the different classes and orders took place several million years ago, there is ample time for evolutionary paths to have diverged and for quite different capacities and mechanisms to have evolved (Macphail, 1982). It follows that the simple appearance of a common trait does not necessarily mean that it evolved as one. Similar traits might have evolved in the separate classes because of similar selection pressures. For valid conclusions to be drawn, it is essential to understand the underlying cognitive mechanisms in detail. In the present chapter we attempt such an analysis for the case of spatial cognition in reptiles.

When it comes to spatial cognition, there is an additional reason to direct special attention to reptiles. Modern interest in spatial learning has it origins in the influential book published by O’Keefe and Nadel (1978) entitled *The hippocampus as a cognitive map* in which it was argued that the spatial competence of mammals, derived in large measure from their ability to form a map-like representation of their environment, and that the cerebral basis of this lay in a brain structure called the hippocampus. A homologous brain structure had been identified in birds, but, although it has been suggested (Rodriguez et al., 2002) that the reptilian medial cortex might serve a similar function, the identification of an equivalent for reptiles, is much less secure. Evidence of refined spatial abilities in reptiles would thus, on the face of things, allow the possibility of testing and developing the hippocampal hypothesis. Are non-hippopcampal reptiles capable of spatial cognition at all?

Reptiles are, of course, capable of moving appropriately through their environments between, for example, feeding grounds, hiding places, and sleeping quarters. This behaviour could, in principle be the product solely of immediate responses to local cues (cf., Crews, and Wilczynski, 1999), but there is plentiful evidence that reptiles are capable of more than that. To give a few example: As we shall discuss later in the chapter, several reptile species of different orders are capable of homing form substantial distances (Ellis-Quinn and Simon, 1989; Krekorian, 1977), or have the ability of returning seasonally to specific locations (Avens and Lohmann, 2003; Sale and Lushi, 2009). In laboratory studies it was shown very early on (by Yerkes, 1901, for the speckled turtle, *Clemmys guttata*, and by Tinklepaugh, 1932, for the common wood turtle, *Clemmys insculpta*), that reptiles can learn to successfully navigate simple mazes. (See Burghardt, 1977, for a full review of early work on reptile cognition.) The capacity of terrapins capacities to learn to navigate T-shaped mazes (*Chrysemys picta picta*, Kirk and Bitterman, 1963) is well established, as is the ability of these animals to show improved learning when subjected to a series of reversals in which the location of the rewarded goal-box is switched from time to time (Holmes and Bitterman, 1966).

It might be argued that the navigational abilities just noted are the consequence of mechanisms quite different from those used by mammals equipped with a hippocampus. An animal that navigates, for example, by following an odor trail, by homing on the basis of a specific landmark, or by learning a particular response pattern, might be regarded as lacking ‘true’ spatial cognition’ of the sort envisaged by the cognitive-map hypothesis. But this argument should be treated with caution, for two reasons. First, of course, we should not prejudge the mechanisms employed by reptiles when they show navigational skills, as that is a matter for experimental analyses of the sort to be described shortly. Second, the notion that mammalian spatial cognition may be mediated by a cognitive map is itself debatable. Bennett (1996), for example, has argued that observations taken to indicate the existence of a cognitive map are susceptible to alternative, simpler explanations. The approach to be taken here, therefore, starts with the simple observation that reptiles (like other animals) are able to find their way about, and assumes that our task is to determine how they come to do it. We begin by discussing long-range navigation, where there is evidence suggesting a role for geomagnetic cues. We will then separately discuss homing behaviour and the role played by celestial cues in this. Finally we will discuss shorter-range navigation, in which response learning and landmark use (perhaps, even, involving the use of some form of cognitive map) appear to be important. All these will be taken as mechanisms that serve to generate the set of abilities that are conveniently labeled as demonstrating ‘spatial cognition’.

The studies to be described use a variety of methodologies, from field observation to controlled laboratory experiment. Long-range navigational systems, in particular are, given their nature, necessarily examined mostly by field observation. In many cases the research is purely observational, and the researchers do not manipulate the conditions the test animal is experiencing. Other studies use quasi-experimental designs, in which researchers manipulate one specific aspect of interest and observe the effects upon the animal in its natural habitat. In this case only the aspect that has been manipulated is under the experimenter’s control. Naturally changing variables (such as the weather) can be taken into account and analyzed, but are not directly controlled by the researcher. In a fully controlled experiment the researcher is ideally able to control all exogenous influences that are acting upon the animal during the experiment. To accomplish this, it is usually necessary to conduct the research inside or in very carefully designed outside experimental apparatuses.

Long-Range Navigation and Geomagnetic Maps

Examples of Long-Range Navigation

Probably the most famous example of reptile long-range navigation is that of sea turtles who travel thousands of miles through the oceans to return from their feeding grounds to their breeding grounds near to where they themselves have hatched (Dutton, Bowen, Owens, Barragan, and Davis, 1999). Leatherback turtles, for example, travel between nesting beaches in tropic and sub-tropic regions (e.g. the Caribbean) and feeding grounds in the North Atlantic (latitudes between the Azores and the United Kingdom; Hays, Houghton and Myers, 2004). Tracking turtle navigation over very long distances has been made possible by satellite technologies which receive information from transmitters attached to the animals’ bodies. When the turtle breaks the surface of the ocean to breathe the transmitter sends information about the turtle’s location, the depth it has dived to, and related issues to a satellite (Morreale, Standora, Spotila, and Paladino, 1996). This technology has enabled a more detailed understanding of the exact nature of turtle migration. For example, Lambardi, Lutjeharms, Mencacci, Hays, and Luschi (2008) tracked nine leatherback turtles that nested near the South African coast and found that they took very different routes, travelling at different speeds and through different areas to reach their destination. In contrast, Morreale et al. (1996) have shown strong relationships between the migration routes used by eight leatherback turtles that nested at the western coast of Costa Rica. These turtles travelled through open waters within a very narrow corridor and rarely dived deeper than 300 meters. Findings such as these are important to aid conservation efforts, as the relevant areas can, for example, be protected from fishery.

Recently, the integration of satellite data and oceanographic information has vastly improved our data interpretation abilities in terms of the interconnectedness of turtle movements and oceanic currents (Sale and Luschi, 2009). Although the sheer distance that these turtles cover poses one enormous challenge to their navigational skills, the constant drift of the oceanic currents poses another. The oceanic drifts are of great importance because, if not taken into account, they can result in severe navigational errors. Girard, Sudre, Benhamou, Roos, and Luschi (2006) found that oceanic currents did pose difficulties for green turtles (*Chelonia mydas*) during navigation in the Mozambique Channel, by interfering with a direct travel route to their goal islands. In contrast, Lambardi et al. (2008) showed that, for example, leatherback turtles drift with the oceanic currents for long periods of time, appearing to use them to aid their movements. On average 73% of the total number of the turtle routes they tracked were determined by currents. The importance of these currents for the movements of the leatherback turtle inevitably leads to the question of how the turtles know how far they have been displaced by a given oceanic drift. In the deep open sea points of reference, such the seabed or the border between currents, which might help the detection of current flow strength, are sparse. While the detection of current flow might be used to some extent (Luschi et al., 2003), it has to be noted that most turtle tracking routes lie in deep waters and inside the core of the currents. As such, flow detection does not appear to be a satisfactory explanation for the exact navigational skills of sea turtles (Sale and Luschi, 2009).

The Geomagnetic Map

One possibility is that the turtles navigate using a geomagnetic map. This means that they use variations in the Earth's magnetic field to orient themselves (Sale and Luschi, 2009). Different locations are defined by unique combinations of magnetic field intensity and inclination which might enable turtles to locate their position in the ocean in relation to a goal. A method that can be used for testing geomagnetic map navigation experimentally, is displacement. This refers to moving the animal away from a goal to which it is motivated to return and tracking its return journey. To test this in a more controlled experimental setup rather than the open sea, the turtles can be transferred to a test arena filled with sea water. Magnets placed around the test arena allow its magnetic field to be manipulated in terms of magnetic intensity and inclination. It is, thus, possible to simulate the magnetic field of any given location in the ocean (Lohmann, Cain, Dodge, and Lohmann, 2001). To determine the swimming direction, the turtles are commonly fitted with a harness around their shells. The harness is, through a rope, attached to a rotatable arm, which is fixed centrally, above the water's surface and attached to a recording computer (figure 1).

Using this kind of setup Lohmann and Lohmann (1994) tested loggerhead sea turtle (Caretta caretta L.) hatchlings on their ability to perceive the inclination of the magnetic field while magnetic intensity was kept constant. Turtles presented with the inclination angle of their natal beach headed eastwards, whereas those presented with an angle only 3° greater headed south-south-west and those presented with a much smaller angle were headed north-east. When presented with a number of other inclination angles the turtles oriented at random.

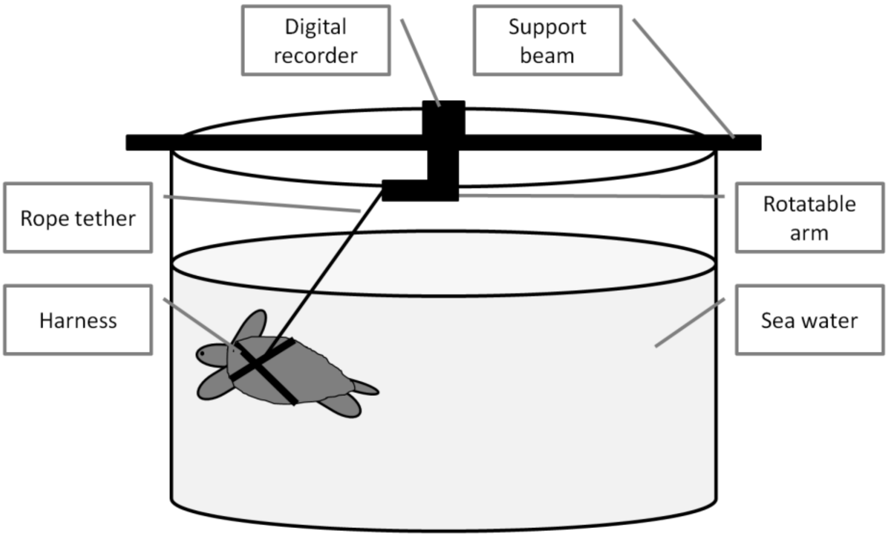

Figure 1. Circular experimental arena, commonly used to test sea turtle swimming orientations, including a turtle wearing a harness which is attached to a rotatable arm and a digital recorder.

Although it is unclear why a specific orientation pattern was seen with only some inclination angles, the results show that loggerhead hatchlings were able to perceive variations in magnetic field inclinations. The authors concluded that these sea turtles might, thus, be capable of determining latitude. In a second study Lohmann and Lohmann (1996) presented loggerhead hatchlings with two different magnetic field intensities emulating opposite ends of the Atlantic Ocean. When presented with a magnetic intensity only encountered in the north the hatchlings headed westwards and when presented with one found only in the south they headed eastwards. This reflects the direction required to keep the turtle within the gyre (a large-scale system of rotating ocean currents) in which they grow up. The differential responses show that loggerhead hatchlings can distinguish between different magnetic field intensities. Based on the findings that loggerhead hatchlings can perceive both the inclination and intensity of the Earth’s magnetic field, the authors concluded that the turtles might, over time, learn the gradients of the magnetic features and, as adults, possess a fully functional, large-scale magnetic map to guide their long-distance migrations. However, testing this hypothesis is extremely difficult.

To test whether loggerhead turtles would be able to distinguish between magnetic fields that actually exist in the ocean along their natural migration routes, Lohmann et al. (2001) presented hatchlings with three different simulated magnetic fields, which can be found at critical turning points along the hatchlings journey. The hatchlings responded to each magnetic field with a different orientation, which shows that they are able to distinguish between naturalistic magnetic fields. The orientations matched the natural migration path in the gyre in which young loggerhead turtles spend the first two to four years of their lives. Keeping within this gyre is essential for the young turtles as the ocean currents leading of the gyre would transport them into areas in which shortage of food supplies or cold temperatures would not allow their survival. It should be noted that none of the hatchlings tested in this study had ever been in the ocean. Thus, the finding cannot be the result of experience with the magnetic fields of relevance but is likely to be an innate response. It is unclear whether hatchlings are equipped from birth with a fully functional geomagnetic map or are simply programmed to swim into certain directions when specific magnetic fields are encountered resulting in later map formation. To test this it would be necessary to examine the behaviour of hatchlings from eggs clutches found at nesting sites in the Atlantic, Pacific and Indian oceans. As different magnetic fields will be crucial for survival for hatchlings from the different regions, it is important to see whether they all respond in the same way or in a manner appropriate to the particular gyre in which they would naturally spend their initial years.

The majority of magnetic field research has tested the behaviour of hatchlings. However, migration mechanisms used by older turtles that navigate towards specific goals might differ from those of hatchlings that only orient towards broad oceanic areas. Lohmann, Lohmann, Ehrhart, Bagley, and Swing (2004) tested juvenile green turtles and found that they adjusted their direction of travel on the basis of a simulated magnetic field: When the field was equivalent to a more northern location the turtles headed southwards and when it was equivalent to a more southern location they oriented northwards. The authors excluded the possibility of navigation on the basis of distant visual cues. This finding suggests that juvenile turtles, too, were able to perceive the magnetic field and used it to navigate back to their original location.

In conclusion, these findings strongly suggest that several sea turtle species are able to perceive geomagnetic forces and use them to guide their navigation towards a goal. This further enables the turtles to perceive their location in relation to a goal, even after passive displacement. Whether the mapping mechanism is inherited or learned remains unclear, but even entirely inexperienced hatchlings can use magnetic field information to guide their journey along a route that they have never travelled before. Furthermore, geomagnetic maps have the advantage of being greatly resistant to natural disturbances and variations in visual patterns, such as might be generated, for example, by changing weather conditions.

Role of Other Cues in Long-Range Navigation

The availability of geomagnetic maps for navigation in a species does not, however, rule out the possibility of other mechanisms, such as visually guided navigation. Avens and Lohmann (2003) tested the preferred swimming direction of loggerhead turtles in a circular arena which made the use of wave direction, olfactory cues and, due to its high walls, the use of non-celestial landmark cues, impossible. On the day following the original direction preference test turtles were tested again in one of three experiments. The first experiment was designed to test the effects of magnetic field manipulation on turtle navigation. Two magnets were fixed to the turtle’s head and shell. In one condition the magnetic field of the magnets was directed downward, for another upward. One control group was equipped with non-magnetic brass bars and another one received no special treatment. The results showed no effect on the turtles preferred orientation direction. The second experiment tested the effects of visual cues on maintaining the preferred orientation. Turtles were fitted either with goggles without lenses or with goggles containing frosted plastic lenses which depolarized sunlight and made locating the sun impossible. A third group received no specific treatment. Again, no difference was found between the groups, with the turtles of all three conditions maintaining their heading in their preferred direction. A third experiment tested the effects of disrupting both magnetic and visual information. All groups of turtles were equipped with goggles with frosted plastic lenses. The first group was additionally fitted with downward pointing magnets, the second with upward pointing magnet and the third group with brass bars. The results showed that while the animals that were fitted with brass bars were able to orient in their preferred direction, those who were equipped with either magnet oriented at random. This suggests that loggerhead turtles have at least two navigational systems, one using geomagnetic maps and another using visual cues which can be relied upon interchangeably when one is unavailable. As the wall of the test arena prevented the turtles from seeing distal landmarks and the inside of the pool did not show any apparent visual features, the authors concluded that the visual cues which the turtles used were likely to be celestial. This suggests that the turtles might have used a celestial compass. Celestial cues, which have been shown to be important for homing in a variety of reptiles, will be taken up next in the context of a discussion of homing.

Homing and the Celestial Compass

Examples of Homing

Several species of reptile are able to return to their home territory after displacement by an experimenter (e.g. terrapins, *Trionyx spinifer, Chrysemys picta* and *Terrapene carolina,* DeRosa andTaylor, 1980; lizards, *Sceloporus jarrovi,* Ellis-Quinn and Simon, 1989; desert iguanas, *Dipsosaurus dorsalis,* Krekorian, 1977; alligators, Rodda, 1984). Krekorian (1977) showed that of 83 desert iguanas 22 successfully homed at least once when displaced between 50 and 400 meters from their territory. The longest homing distance observed in this study was 174 meters. In later work, Ellis-Quinn and Simon (1989) found that displacement direction did not affect the homing performance of Yarrow's spiny lizards (*Sceloporus jarrovi*). This suggests that to return home the lizards did not simply move in one stereotyped compass direction. Furthermore, their return journey took a relatively direct route. This study also found that the age of the lizards had a significant effect on their homing success. Adults were able to return home after a 200-meter displacement whereas two-month-old juveniles only returned if the displacement was no further than 50 meters. Within the group of adult animals, a significantly larger number of older individuals returned compared to the number of one-year-old individuals. A possible explanation for this age difference is that younger animals are less familiar with the more distant areas around their home range. Another possibility is that younger animals are less attached to their home range and, as such, are less motivated to return (Ellis-Quinn and Simon, 1989). Further evidence from experiments with chelonian revealed that three terrapin species showed significant homeward orientation after being displaced on sunny days but not when they were displaced on cloudy days (DeRosa and Taylor, 1980). This suggests that the sun may have played a critical part in their navigation.

Role of Celestial Cues

Although these studies show that different reptile species are able to return to their home range from distant locations to which they have not ventured themselves, only the terrapin study provides a clue about the cognitive processes underlying their behaviour. Recent research has suggested that time-compensated celestial compasses may control some reptilian navigational behaviour. There are two possible versions of a celestial cue compass: A sun-azimuth compass and a sky-polarization compass (Beltrami, Bertolucci, Parretta, Petrucci, and Foà, 2010). The sun's azimuth is the angle of the point where a vertical line dropped from the position of the sun crosses the horizon with the point that the animal is intending to travel to. For an animal to use this, it is important that its internal circadian clock has experience of the time of day in a specific place and can associate this with the resulting azimuth patterns. The downside of this compass is that the sun itself needs to be visible for it to function, which is often not the case for small lizards that live under leaf litter or vegetation. The second variation of the celestial compass is not affected by this shortcoming and only requires a small area of blue sky to be visible. Sunlight gets partially polarized when passing through the atmosphere. The polarization pattern of the sky varies predictably with the position of the sun as it produces a symmetrical e-vector (beam of polarized light) pattern which relates to the sun's position (Beltrami et al., 2010). A common method for testing whether navigational behaviour is guided by the movements of the sun is to maintain the animal in an artificially shifted time schedule which varies from the actual time at the research location. This procedure is referred to as clock-shifting and the induced time difference is most commonly six hours. After the clock-shift adaptation which normally lasts between three and seven days, the animal is tested outside, so that the actual position of the sun indicates a different compass direction when transferred to the animal's internal clock. A 6 hour clock-shift corresponds with a 90°shift of the sun's position and, thus, should lead to a 90° direction modification in a clock-shifted subject. In other words, if you see the sun in one location and you think it is 12 pm the sun's position would indicate the compass direction south. If, however, you assume it is 6 pm the sun's position would indicate the compass direction west.

Newcomer, Taylor and Guttman (1974) trained two species of water snakes (*Natrix sipedon* and *Regina septemvittata*) to move in a certain direction. After clock-shifting the animals by six hours the water snakes produced the expected 90° orientation modification. This supports the hypothesis that they used celestial cues in combination with a time-compensating internal clock. Similar results have been observed in painted turtles (*Chrysemys picta*; DeRosa and Taylor, 1978) and fringe-toed lizards (*Uma notate*; Adler and Phillips, 1985). Research with crocodilians has revealed that juvenile American alligators use solar and stellar cues to guide navigation (Murphy, 1981). Solar navigation seemed to work in a similar manner to the studies above. However, the stellar compass appeared to be independent of any clock-shift effect and is likely to rely on the stellar pattern alone. These results show that reptiles from a number of different reptile orders are capable of using celestial cues to guide navigation.

The importance of celestial cues for navigation gives rise to the question as to which sensory organ mediates celestial cue perception. There is evidence to suggest that some amphibians can use extra-ocular photoreceptors (light-sensitive cells at the tops of their heads) for orientation (Taylor and Adler, 1978). This, in combination with the relatively close phylogenetic relationship between amphibians and lizards coupled with similarities in the relevant brain areas, prompted researchers to investigate the role that the lizard parietal eye plays in their spatial navigation (Ellis-Quinn and Simon, 1991). The parietal eye is a light-sensitive structure located at the top of the lizard's head. It responds to illumination and cessation of light and transmits the received information to the pineal gland in the brain (Engbretson and Lent, 1976). A number of studies have investigated the role that the parietal eye plays in navigation and have shown that it is likely to mediate the use of celestial cues (Beltrami et al., 2010; Ellis-Quinn and Simon, 1991; Foà et al., 2009; Freake, 1999; Freake, 2001; Phillips Deutschlander, Freake, & Borland, 2001).

Ellis-Quinn and Simon (1991) displaced three groups of the Yarrow's spiny lizard (*Sceloporus jarrovi*) 150 meters from their home range and tracked their movements. In the experimental animals the parietal eye and surrounding area was covered with black paint whereas for a sham-treated control group only the area surrounding the parietal eye was covered. A second control group consisted of untreated animals. Sham-treatment is an experimental manipulation that is considered to have the same degree of invasiveness as the experimental treatment but without affecting the areas of interest. The results showed that all untreated and all sham-treated lizards returned home within two days, whereas none of the experimental animals returned successfully or demonstrated a homeward orientation. Thus, the authors concluded that the parietal eye is a critical receptor for celestial cues and is necessary for successful homing in the Yarrow’s spiny lizard. In a similar experiment, Freake (2001) displaced Australian sleepy lizards (*Tiliqua rugosa*) and manipulated the use of the lizards’ parietal eye. The parietal eye was either fully covered or fitted with a sham-cover, which only covers the area around the parietal eye. The subjects showed random orientation only when the parietal eye itself was covered and not when the surrounding area was (Freake, 2001). These results suggest that the parietal eye is necessary for successful homing behaviour in Australian sleepy lizards. Similarly, Foà et al. (2009) showed that ruin lizards (*Podarcis sicula*) were able to learn to navigate to a fixed position in an outdoor Morris water-maze (figure 2). A Morris water-maze is a water filled arena in which a platform is positioned just below the water’s surface at a specific location. This platform functions as the goal. The water is rendered opaque so the animals have to use distal cues to swim to the correct position. Any possible odor cues that an animal might leave during exploration of the maze are thus dispersed by the water and can be excluded as possible mechanisms underlying a successful performance.

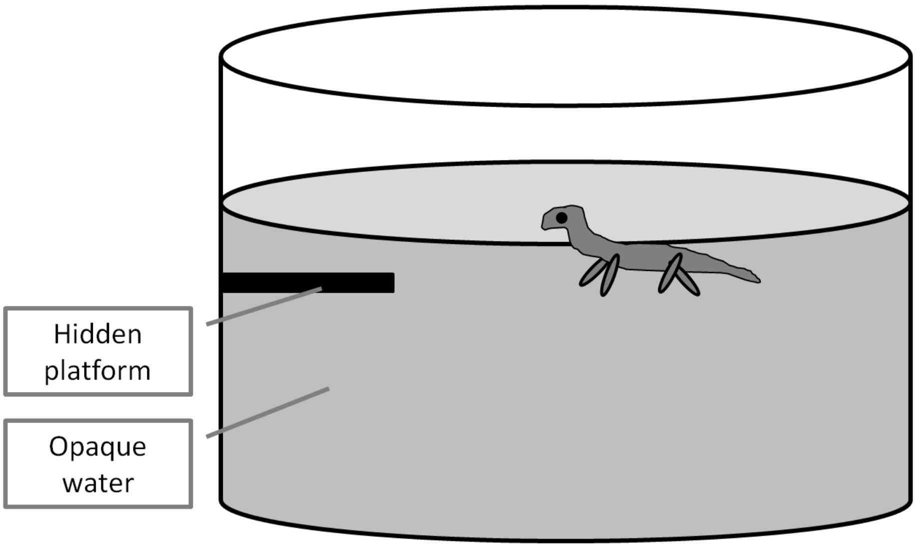

Figure 2. Morris water-maze with a platform hidden under the surface of water which has been rendered opaque.

Foà et al. (2009) surrounded the water-maze with a high wall which prevented the lizards from perceiving any environmental cues, except the sky. The lizards were subjected to 6-hour clock-shifts, either forward or backward, and, when tested under normal daylight conditions, showed response directions in an angle according to the time variation. A further experiment was run, in the same experimental setup, in which either the lizards' parietal eye (experimental treatment) or the area around the parietal eye (sham-treatment) was covered in black paint (Foà et al., 2009). The results showed that only the sham-treated lizards were able to orient to the platform, while the lizards with covered parietal eyes searched for the goal at random. However, while the evidence for the importance of the parietal eye in the perception of celestial cues is undeniable, none of the studies described above provide proof that the parietal eye alone is sufficient for the perception of celestial cues as the lateral eyes of the animals were not covered during the experiments.

It is clear that celestial cues play an important role in navigation for many lizard species. However, these studies do not reveal the mechanism controlling these behaviours. The lizards could have used navigation based on the sun-azimuth or sky-polarization in these tasks. Evidence for the use of a sky-polarization compass was provided by Freake (1999) who trained sleepy lizards (*Tiliqua rugosa*) under fully controlled, artificial polarized light conditions in a round indoor arena. The lizards were required to orient along a single e-vector axis, a task which they successfully learned. This result reveals that lizards are able to navigate using a sky-polarization compass. Further evidence for the use of such a compass was provided by Beltrami et al. (2010) who examined whether ruin lizards (*Podarcis sicula*) were able to perceive artificially produced e-vector directions. The lizards learned to follow the direction of a single e-vector to guide them to one of two hidden goals at either side of an indoor Morris water-maze. After a 90° rotation of the e-vector the lizards responded by adjusting their search behaviour and followed the altered e-vector direction. In a second experiment Beltrami et al. (2010) painted over the parietal eye of the lizards. As a consequence the lizards were no longer able to find a platform and appeared completely disorientated. This shows that the parietal eye is in fact capable of perceiving polarized light. However, these results do not exclude the possibility that there is also a sun-azimuth compass which might also be mediated by the parietal eye. Whatever is true for long-range homing, it seems clear that other mechanisms come into play when reptiles need to find home from a short distance. For example, painted turtles (*Chrysemys picta marginata*) when displaced a mile from their home pond became disorientated and failed to find their way back (Emlen, 1969). However, when released 100 meters from home they were able to return quickly, and on a direct route. Further, they were able to do this on overcast as well as sunny days. The turtles appeared to be using landmarks, such as the edge of a wood near the home pond, to guide their homeward passage. Landmark use and other processes effective in short-range navigation have been subjected to extensive study with captive animals in laboratory procedures, and we turn to a discussion of this work in the next section.

Short-Range Navigation

Although the long-range navigational feats of reptiles are especially likely to attract our attention, their biological success will depend equally on their ability to find their way about on a smaller scale. For example, within the home range, they may need to be able to find their way back to their home pond after foraging elsewhere; and when foraging they may need to move efficiently from one site to another, avoiding those in which supplies have been depleted, and moving on to new ones. In this section of the chapter we discuss both these skills – learning to find a single goal ("win-stay" behaviour) and learning to move around from one to another ("win-shift" behaviour).

In recent years much work on these topics has made use of experimental studies of captive animals run in an apparatus (e.g., a maze) in which visual and other cues can be directly manipulated. In navigating around such apparatus, one strategy, generally referred to as response-based learning, is to learn a sequence of responses (for example, turn left, then turn right; or always turn right), which would allow the animal to reach its goal independent of external cues. But such a method would be efficient only under very stable conditions and would be unreliable in an ever-changing natural environment. Experimental studies have established that external (especially, visual) cues may play an important role. It is worth noting at this stage that they may function in different ways. Most obviously, a single, salient local cue, close to a goal, could function as a beacon (or landmark) on which an animal can home. But distal cues, positioned at a distance from both the animal and the goal (in experimental studies these are often furnishings and decoration of the test room referred to as room cues or extra-maze cues), can also serve to guide behaviour. In this case a place may be defined not by its relation to a single landmark but in terms of its spatial relation to a range of them. Navigation using such cues has been demonstrated for mammals and birds and is sometimes referred to as involving a cognitive map (although the appropriate use of this term is far from agreed, e.g., Bennett, 1996; Mackintosh, 2002). For our purposes the critical feature is that the controlling aspect of the environment is a configural array of cues rather than a single cue, making it possible for successful navigation to persist despite the removal of a single landmark.

Short-Range Navigation to a Goal

Experimental studies of short-range navigation in reptiles have generated a range of different results for different species belonging to different orders. In a study of two closely related lizard species (*Acanthodactylus boskianus* and *Acanthodactylus scutellatus*), Day et al. (1999) provided the animals with both a local, beacon cue and distal, extra-maze cues. Neither species preferentially attended to either distal or local cues. The lizards of both species rarely approached the goal directly, and seemed to find it through trial and error search. This led the authors to conclude that spatial learning processes in these animals were fundamentally different from those observed in mammals and birds.

In contrast, other reptile species appear to be readily able to learn to navigate to a specific goal using local visual cues. Holtzman, Harris, Aranguren, and Bostocks (1999) released corn snakes (*Elaphe guttata guttata*) into an arena that contained one true hiding hole (the goal) and seven pretend holes that appeared identical to the goal, until close inspection revealed that they were impossible to access. One side of the arena contained a large white card square, and other visual cues were excluded by surrounding the arena with a black curtain. In these circumstances the snakes proved capable of rapidly learning the position of the goal, presumably by making use of knowledge of its position with respect to the white card.

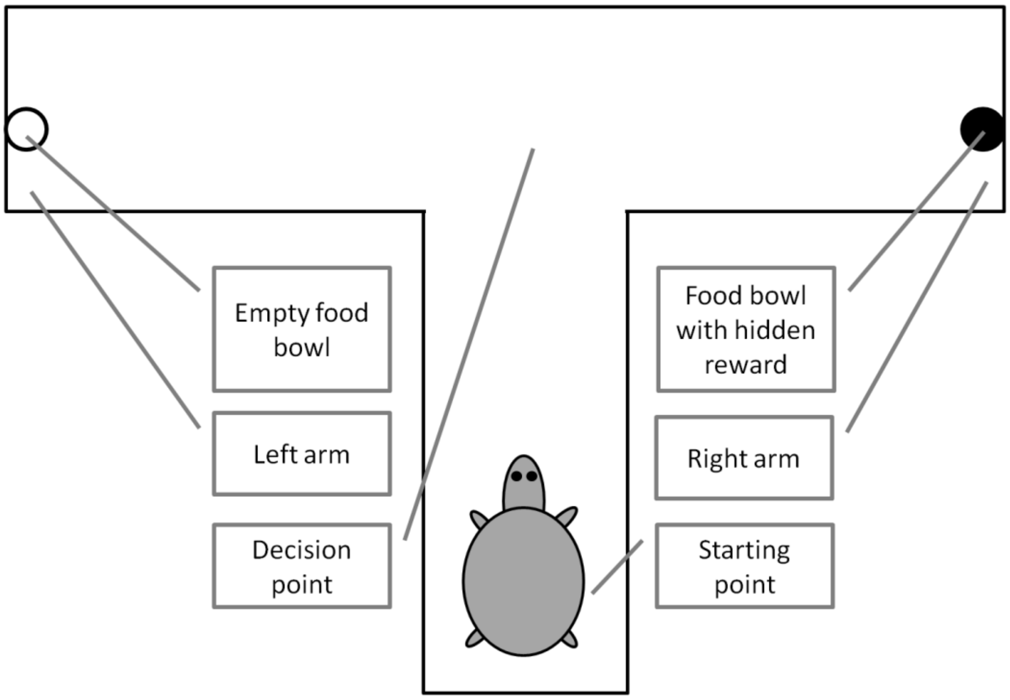

Figure 3. A T-shaped maze where food rewards can be hidden in either left or the right arm.

Studies of spatial learning in chelonia (turtles, terrapins and tortoises) reveal evidence of navigation based on a configuration of distal, extra-maze cues. López et al. (2000) showed that the terrapin (*Pseudemys scripta*) could learn to approach a specific location in a T-shaped maze (figure 3), regardless of the starting position, even when no single cue served as a beacon. Excluding the extra-maze cues by surrounding the maze with a curtain disrupted performance, but performance remained good if the curtain obscured only some of these cues. The authors suggested that the terrapins were navigating using a cognitive map of the sort that is postulated in mammals and birds. Later findings revealed that the terrapins were also able to navigate using a beacon, and that the mechanism used depended on the cues that were available.

López et al. (2001) trained one group of terrapins to navigate to a specific goal with only distal cues provided. A second group received the same distal cues and an additional beacon cue that was located close to the goal. Both groups successfully learned to navigate to the goal. Test trials revealed that the distal cue group appeared to make use of a cognitive map-like representation whereas the beacon group did not use the distal cues and instead used the single beacon to locate the goal. This pattern of results suggests that reptilian learning and memory capabilities, in this species at least, may closely parallel those observed in mammals and birds.

To investigate this further, López, Vargas, Gómez, and Salas (2003) examined the role that the medial cortex plays in the navigation of this species. As we have noted, it has been proposed that the function of this structure might parallel that of the mammalian hippocampus. López et al. (2003) found that surgically induced lesions to the medial cortex of terrapins (*Pseudemys scripta*) resulted in a qualitative change in performance in an open field task in which the animals learned to navigate to a goal in an open arena on the basis of surrounding distal cues. Intact terrapins readily learned to navigate in the arena, but after lesioning, performance fell. With continued post-operative training, the lesioned animals were able to learn to navigate to the correct location and their performance eventually equaled their pre-operative level and the level shown by sham-operated animals. The authors then presented the terrapins with a series of test trials in which the extra-maze cues were either fully or partially obstructed. Sham-operated animals continued to perform well when the extra-maze cues were obscured only partially, whereas lesioned animals did not, suggesting that their new solution was not the result of the map-like strategy used by the control subjects. López et al. (2003) argue that this pattern of results parallels that obtained in mammals and birds and further suggest that the medial cortex is essential for cognitive map formation and thus performs a similar function to the mammalian/avian hippocampus.

Spatial Win-Shift Tasks

Although different in their details, the experiments described in the previous section have in common that they all require the subject to remember and travel to one goal location. This may be described as involving a ‘win-stay’ paradigm, and it is the form of spatial learning that has been most studied in reptiles (Wilkinson and Huber, in press). But just as interesting and important is the ‘win-shift’ paradigm. In this setup animals are required to retrieve rewards from a number of different locations and, importantly, not to revisit the same location within a trial. Given the behavioural ecology of many reptile species, this sort of task might be considered as being in some sense more natural – many reptiles might need the ability to remember places that have previously been visited in order to ensure that they do not return to an area in which a food source has been depleted. Although solving tasks of either type requires spatial learning and long-term memory, win-shift tasks, additionally, pose high demands on working memory.

The classic example of a win-shift task is learning the radial-arm maze (figure 4). This maze was originally developed for rats (Olton and Samuelson, 1976). It has a central area from which eight identical arms radiate, like spokes on a wheel. Hidden food is available at the end of each arm, so that, as the trial progresses a subject has to remember which arms it has visited to know whether the food source has already been depleted. A well trained rat will visit each arm to collect the food, and rarely return to arms that it has previously visited, exhibiting an ability to discriminate among the various spatial locations, and remember which places have been visited on a given trial. The procedure provides an excellent test of an animal’s spatial learning capacities and its working memory and is easy to adapt for use with other species. Wilkinson, Chan, and Hall (2007) examined whether a red-footed tortoise (*Geochelone carbonaria*) could learn to successfully navigate in an eight-arm radial maze. The tortoise showed reliable, above-chance performance, preferentially choosing novel, and therefore baited, arms rather than returning to arms previously visited within a trial. Tests ruled out the use of olfactory cues from either the bait, or from the avoidance of odor trails which the tortoise could potentially have left. The tortoise did not exhibit any response-based strategies but rather she appeared to use the distal room cues for navigation. These results support the findings of Lopez et al. (2001, 2003) and suggest that, in spite of differences in brain structure, chelonia possess spatial learning abilities comparable to those observed in mammals.

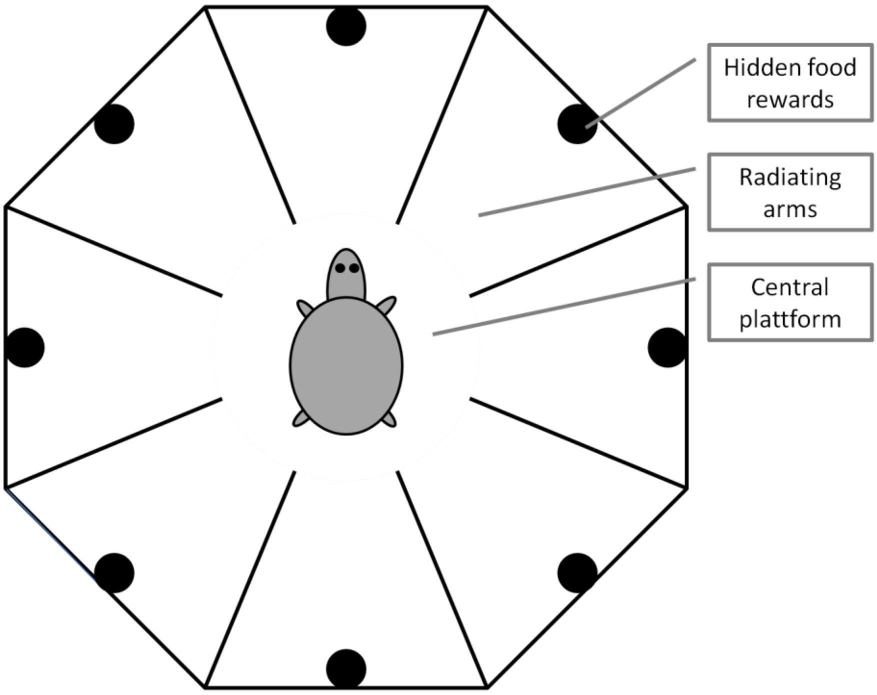

Figure 4. Eight-arm radial maze where food rewards are hidden in each arm. All arms can be accessed from a central platform.

In a second study, Wilkinson, Coward, and Hall (2009) trained the tortoise with the maze surrounded by black curtains which obscured the extra-maze room cues. Small colored shapes attached to the curtains were evidently unable to support performance of the type seen when the animal had access to the full set of cues supplied by the room as in these circumstances the tortoise adopted a novel, but simple and highly successful strategy – that of entering the arm next to the one she had just left. This behaviour was not a fixed pattern, as the direction of movement (i.e. turning left or turning right), although consistent within a trial, varied from one trial to the next. In a follow-up experiment the curtain surrounding the maze was removed and the tortoise had access to all of the cues available in the experimental room. The use of the turn-by-one-arm strategy then reduced to chance levels and the tortoise apparently used the visual cues from the room to navigate. These results have in the meantime been replicated with additional red-footed tortoises (Mueller, Wilkinson, Huber and Hall, in preparation) and, thus, appear to be a general trait of red-footed tortoises and not specific to the one individual used by Wilkinson et al. (2009).

Such variability in spatial behaviour suggests the presence of two processes that control navigation in the tortoise. One appears to be based on visual cues and to be similar to the cognitive-map mechanism employed by rats (Olton & Samuelson, 1976); the second mechanism involves a response-based strategy similar to that observed in Japanese fighting fish (Roitblat, Tham & Golub, 1982). These experimental findings indicate that when tortoises navigate in a situation with poor environmental cues they use the response-based strategy, but when more cues are available they switch, and navigate using the surrounding visual cues.

To examine whether the use of response-based strategies under poor cue conditions is the result of the behavioural ecology of the red-footed tortoise, or is also found in other reptiles, Mueller, Wilkinson, Hall, and Huber (submitted) investigated the performance of the jeweled lizard (*Lacerta timon lepida*) in an eight-arm radial maze. This species was chosen because it differs from the red-footed tortoise in terms of diet (largely insectivorous vs. largely frugivorous), habitat (temperate vs. tropical), and evolution (squamate vs. chelonia). These species have not shared a common ancestor for around 225 million years (Macphail, 1982). Therefore, the occurrence of a response-based strategy would suggest that this ability is not specific to chelonia and may be something common to all reptiles. The results show that the jeweled lizard consistently navigated the maze using the turn-by-one-arm behaviour observed in the tortoise by Wilkinson et al. (2009). This finding supports the idea that this navigational mechanism could be common to all reptiles, though further research should investigate this in other reptile orders.

Summary and Conclusions

Reptiles, birds and mammals all evolved from a common amniotic ancestor and it is likely that they share behavioural as well as morphological traits. Equally, since this putative ancestor lived around 280 million years there is ample time for very different capacities and mechanisms to have evolved. To fully understand the evolution of cognition it is necessary to examine cognitive processes in reptiles in a similar framework to observations and experiments conducted with mammals and birds. This review has attempted to provide an overview of the advances in our understanding of a specific area of reptile behaviour over the last 30 years: the study of spatial cognition.

Spatial cognition is considered an essential survival tool for almost all species (Day et al., 1999) as it allows navigation through complex natural environments (Reber and Reber, 2001). It supports efficient passage between feeding grounds, hiding places, and sleeping areas and is thus highly advantageous in evolutionary terms. Recent research in spatial learning has focused on the hippocampus, the brain area which is thought to control spatial navigation in mammals (O'Keefe and Nadel, 1978). A homologous brain structure had been identified in birds, and it is possible that in reptiles the medial cortex serves a similar function. Thus, evidence of complex spatial abilities in reptiles can inform us about the evolution of spatial learning and the brain processes which underlie it.

When compared to the virtual explosion of spatial research that has been conducted with the other amniotes over the last 30 years, the lack of progress in reptile research is striking. However, the data that are available suggest that the investigation of this cognitive process in reptiles is a valuable and productive exercise. This review has presented evidence which reveals that some species of reptile can travel toward a goal that is thousands of miles from their current position. To do so they appear to use geomagnetic maps. The role that learning plays in this navigation is currently unclear; however, it is known that some responses to changes in magnetic fields appear to be innate. However, experiments with juvenile turtles reveal that these cues can be used in combination with celestial cues (Avens and Lohmann 2003).

Celestial cues also appear to be used in a similar manner in the homing behaviour of a number of reptile species from three different orders. This contrasts to the results of experiments investigating short-range navigation in laboratory setting, which have produced differing results in the different reptile orders. Some research suggests that the medial cortex serves a similar function to the mammalian hippocampus, whereas other work suggests that there may be qualitative differences between reptiles and mammals. This raises a number of interesting questions about the relative roles of behavioural ecology and evolutionary history in shaping cognitive abilities.

The investigation of cognitive abilities in reptiles is itself an important endeavor. The increased understanding of reptile spatial cognition provides a solid basis on which to design experiments for the much less well understood areas of reptile cognition (e.g. social or visual). Further, the knowledge gained from this research has important implications for the development of welfare and conservation programs directed towards reptiles.

From a more theoretical perspective; in order to reach a full understanding of the evolution of cognition it is essential that we investigate the similarities and differences in the cognitive processes underlying the behaviour of all amniotes. The study of spatial cognition in reptiles has revealed that this is a fruitful approach. However, our knowledge is far from complete. In the words of Alerstam (2006) “After longstanding and intensive migration research, we are still far from a fundamental understanding of animal navigation, and the emerging picture is complex and intricate” (p. 791). Animal cognition research is now at a stage where the mechanisms underlying some of the cognitive behaviour of mammals and birds are well understood. It is now time to invest similar attention to the cognitive abilities of reptiles.

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Chapter 3: Radial-arm maze navigation of the red-footed tortoises (Geochelone carbonaria)

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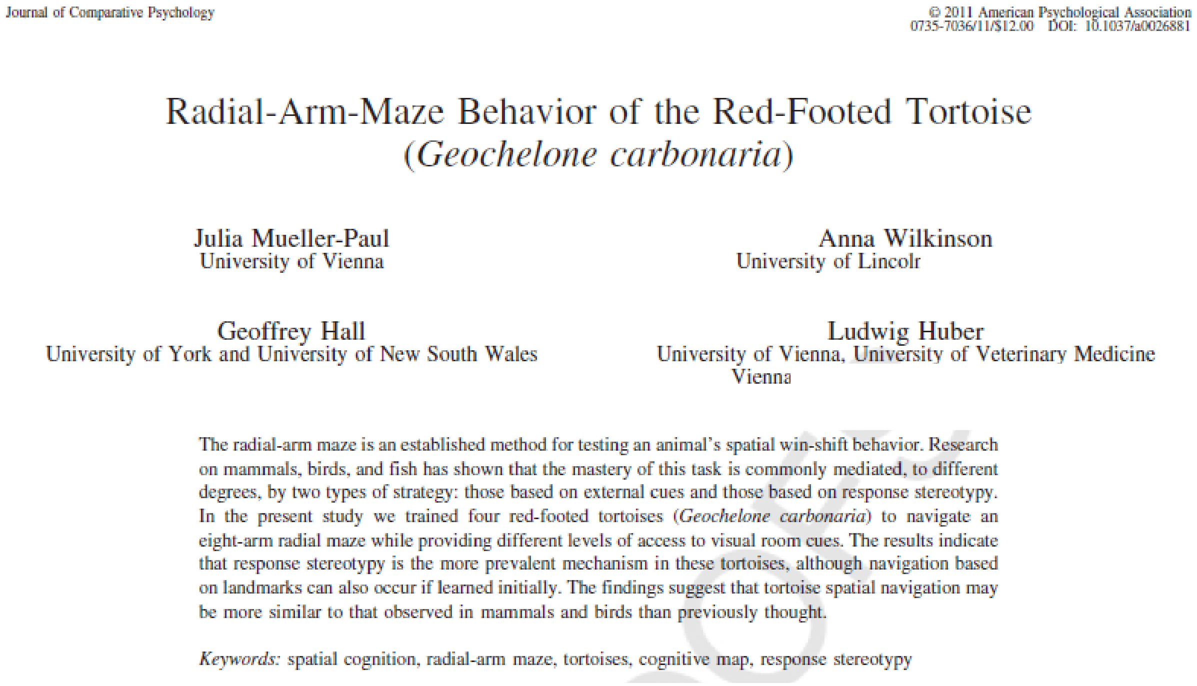

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Radial-Arm-Maze Behavior of the Red-Footed Tortoise (*Geochelone carbonaria*)

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The radial-arm maze is an established method for testing an animal's spatial win-shift behavior. Research on mammals, birds, and fish has shown that the mastery of this task is commonly mediated, to different degrees, by two types of strategy: those based on external cues and those based on response stereotypy. In the present study we trained four red-footed tortoises (*Geochelone carbonaria*) to navigate an eight-arm radial maze while providing different levels of access to visual room cues. The results indicate that response stereotypy is the more prevalent mechanism in these tortoises, although navigation based on landmarks can also occur if learned initially. The findings suggest that tortoise spatial navigation may be more similar to that observed in mammals and birds than previously thought.

Keywords: spatial cognition, radial-arm maze, tortoises, cognitive map, response stereotypy

Abstract

The radial-arm maze is an established method for testing an animal's spatial win-shift behaviour. Research on mammals, birds and fish has shown that that mastery of this task is commonly mediated, to different degrees, by two types of strategy: those based on external cues and those based on response stereotypy. In the present study we trained four red-footed tortoises (*Geochelone carbonaria*) to navigate an eight-arm radial maze while providing different levels of access to visual room cues. The results indicate that response stereotypy is the more prevalent mechanism in these tortoises, although navigation based on landmarks can also occur if learned initially. The findings suggest that tortoise spatial navigation may be more similar to that observed in mammals and birds than previously thought.

Introduction

The ability to orient around complex environments (an aspect of spatial cognition, Reber & Reber, 2001) is considered an essential survival tool for almost all species, as it allows efficient movement between feeding grounds, hiding places, and sleeping areas (Day, Crews, & Wilczynski, 1999). Despite the universal nature of spatial cognition, its study has largely focused on the behaviour of mammals, birds and fish. This has led to a fair understanding of their capabilities (Bond, Cook, & Lamb, 1981; López, Bingman, Rodríguez, Gómez, & Salas, 2000; Vargas, López, Salas, & Thinus-Blanc, 2004). However, to gain a clear understanding of the evolution of vertebrate cognition it is important to examine this ability in reptiles (Mueller, Wilkinson, & Hall, 2011; Wilkinson & Huber, in press). The amniotes (mammals, birds, and reptiles) have common evolutionary roots and can, therefore, be expected to share certain behavioural traits and capabilities. Yet, as the evolutionary split between the different classes took place several million years ago, there is ample time for quite different capacities and mechanisms to have evolved (Zardoya & Meyer, 2001). Thus, the exploration of differences and similarities among the amniotes is likely to provide insight into the evolution of spatial cognition.

Several different methods of spatial orientation have been identified in mammals, birds and fish. Some strategies rely on use of external stimuli as the basis for orientation. One example which is commonly seen in mammals and birds is the use of a distinctive cue located near the goal as a beacon on which to home. Another is the orientation based on an array of different *landmarks* in relation to each other. In this case no one cue needs to be located near the goal. Landmark-use is considered the dominant orientation strategy in mammals and birds (O'Keefe & Nadel, 1978). Another, quite different mechanism involves the development of a *response strategy,* in which a goal is reached by performing a specific sequence of responses – for example, an animal may show a stereotyped patter of always turning left at a choice point. With a strategy of this kind, no external cues are needed to reach the goal successfully. A potential disadvantage of such a strategy may be that it might imply an inability to adapt to a changing external environment. It has been argued response stereotypy generally appears in conjunction with other mechanisms (Bond et al., 1981). Strategies based on external cues will impose a load on the animals' memory (Dale & Innis, 1986), something that could be alleviated if such strategies were supplemented by the development of effective stereotyped response patterns.

Spatial navigation in chelonia (turtles, terrapins, and tortoises) has been a topic of interest for over a century. Early studies demonstrated a general ability to solve basic spatial problems such as multiunit and T-mazes (for a review see Burghardt, 1977); later studies have focused on the mechanisms underlying spatial learning (for a review see Mueller et al., 2011). For example, López et al. (2000) trained the terrapin *Pseudemys scripta* to approach a specific location in a T- maze. They found that, regardless of their starting position, the terrapins successfully used a configuration of distal cues to locate the goal. Partial obstruction of the cues did not negatively affect performance. However, when the cues were completely obscured a drop in performance was observed. Similarly, López et al. (2001) trained two groups of terrapins to navigate to a specific goal and showed that they could use either landmark-based representations or a single beacon, depending on cue availability during training. These findings suggest a close resemblance between the spatial learning mechanisms observed in chelonia and those seen in mammals and birds.

Most research on spatial cognition in reptiles has used tasks requiring navigation towards a single goal (win-stay tasks). Win-shift tasks in contrast require the animal to move around from one goal to another, and to retrieve rewards from a number of different locations that must not be revisited within a trial. The classic test of this is the radial-arm maze (Olton & Samuelson, 1976), which has been used extensively in the study of rat spatial navigation. Rats have been found to be extremely successful in learning to choose the novel arms of a radial mazes (Olton, Collison, & Werz, 1977; Olton & Samuelson, 1976). It appears that their primary orientation mechanism is a memory strategy relying on extra-maze cues (Suzuki, Augerinos, & Black, 1980; Olton & Collison, 1979). This technique seems likely to impose a substantial cognitive load in that the animals must not only form a long-term representation of the relation of the goals to a range of extra-maze cues, they must also use working memory to hold information about which arms have recently been visited. To adopt stereotyped response patterns would ease this load, and map-like strategies can be accompanied by secondary response stereotypy (Dale, 1986; Dale & Innis, 1986) which can take different forms, ranging from a weak tendency towards turning into every other arm to a stable succession of one-arm turns (Suzuki et al., 1980). Whether or not response stereotypy is shown seems to depend on factors such as the cost of errors (Yoerg & Kamil, 1982), the availability of extra-maze cues (Suzuki et al., 1980), the experimental setup (Magni, Krekule, & Bureš, 1979), and the number of trials already experienced during a session (Roberts & Dale, 1981).

Radial maze studies with other species have produced varying results, In some studies pigeons have been found to have success rates well below that of rats and to rely strongly on response stereotypy (Bond et al., 1981; Olson & Maki, 1983; Walcott, 1996); others have found them to be proficient when provided with an appropriate setup (Roberts & Van Valdhuizen, 1985). The Siamese fighting fish (*Betta splendens*) studied by, Roiblat, Tham and, Golub (1982) were able to solve an eight-arm radial maze successfully. The fish showed a strong tendency towards the stereotyped response of sequentially visiting adjacent arms, but this was not sufficient to explain the high levels of performance they achieved. The authors suggested that a memory-based mechanism acted to supplement the behaviour supported by the response strategy component. Hughes and Bright (1999) report an interesting dissociation in two intertidal fish species (*Spinachia spinachia & Crenilabrus melops*). Both species showed response stereotypy when no visual cues were provided. However, a second group of each species, which was provided with spatial cues in the form of colored tiles, navigated using a memory-based strategy.

To our knowledge only three studies have been conducted to date to examine the mechanisms underlying radial-arm maze behaviour in reptiles. A single jeweled lizard (*Lacerta lepida* studied by Mueller, Wilkinson, Hall, & Huber, submitted), was found to display the stereotyped response pattern of emitting a sequence of turns by one arm. A more complex behaviour pattern has been observed in a red-footed tortoise (*Geochelone carbonaria*). Wilkinson, Chan, and Hall (2007) found that the tortoise learned to navigate an eight-arm radial maze achieving a level performance only slightly below that shown by rats and comparable to that of pigeons (Bond et al., 1981). The tortoise did not exhibit any response stereotypy and its performance was not based on olfactory cues. Rather, navigation appeared to rely on a memory strategy based on extra-maze cues. In a follow-up study the same tortoise was trained with a curtain surrounding the maze that obscured all room cues; the only extra-maze cues available were four cut-out shapes pinned to the curtain (Wilkinson, Coward, & Hall, 2009). In this sparse environment the tortoise changed its strategy and began to use a stereotyped response pattern. It turned sequentially into adjacent arms and rarely changed direction during a trial. However, some flexibility was shown in that the turning direction varied between one session in the maze and the next. This stereotyped pattern persisted when the cues were removed leaving a black curtain, but was abandoned when the curtain itself was removed giving visual access to the extra-maze cues of the room.

The results of Wilkinson et al. (2007, 2009) suggest that the tortoise's primary response strategy may be response-based under poor cue conditions, but that a landmark-based strategy can appear when a rich cue environment is provided. Such a clear separation between mechanisms (which is uncommon in rats; Dale & Innis, 1986) might indicate the existence of two separate processes that control navigation in the tortoise. These conclusions, however, are based on the behaviour of just one individual. The present set of studies sought to replicate and extend the findings of Wilkinson and colleagues. In particular, we examined whether learning to navigate around a radial arm maze was an ability that was common to all red-footed tortoises and we sought to determine the ability of the tortoise to switch from one method of solution to another according to circumstances.

Experiment 1: Can red-footed tortoises learn to navigate a radial-arm maze?

Introduction

The aim of this study was to replicate the basic radial-maze procedure employed by Wilkinson et al. (2007) but to test the performance of more animals to establish the generality of the effects observed.

Methods

Subjects

Four juvenile red-footed tortoises (*Geochelone carbonaria*), Esme, Molly, Quinn, and Emily took part in the study. Their plastron lengths were 12.5, 12.5, 11, and 10 cm respectively.

They had a minimal age of 3 years at the beginning of the experiment. The tortoises' sex was unknown, as this species only develops unambiguous sexual dimorphic traits closer to sexual maturity. All four tortoises were captive bred and purchased from licensed pet shops in Austria. The tortoises were housed as a group in a 120 x 70 cm enclosure, at 28 +/- 2°C and approximately 60% humidity, with permanent access to fresh water, shelter, UV light, and heat lamps. During experimental sessions the tortoises were rewarded with small pieces (approximately 0.5 x 0.5 cm) of preferred fruit and vegetables, such as strawberry, sweet corn, and mushroom. They were fed with a variety of less preferred food types, such as apple, pear, and cucumber, in their enclosure after training. In accord with standard husbandry practice they experienced one day a week without food. None of the animals was experimentally naïve (see Wilkinson, Künstner, Mueller, & Huber, 2010; Wilkinson, Mandl, Bugnyar, & Huber, 2010; Wilkinson, Mueller, & Huber, submitted) but they had never previously been involved in a maze task.

Apparatus

The apparatus was an eight arm radial maze (*Figure 1*) with 14-cm-high opaque, plastic walls. The maze floor was covered with grip-ensuring rubber lining and the whole maze was covered by a 1.5 cm^2 wire mesh. 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. This arm shape was used to ensure that the tortoises could turn easily within the arm. A small, opaque, yellow food bowl (diameter 3cm x height 1.5cm) was located centrally at the end of each arm. The rim of the bowl prevented the tortoises from seeing the reward until they had fully entered the arm. The maze was not cleaned thoroughly between trials. This is because multiple arms are entered during each trial, and thus, leaving the maze with many odour cues increased the difficulty of identifying odour information from the present trial. It was thought that this would decrease the value of using odour trails as cues. In the rare cases that the animals defecated or urinated in the maze this was removed and affected area was cleaned after the trial was completed.

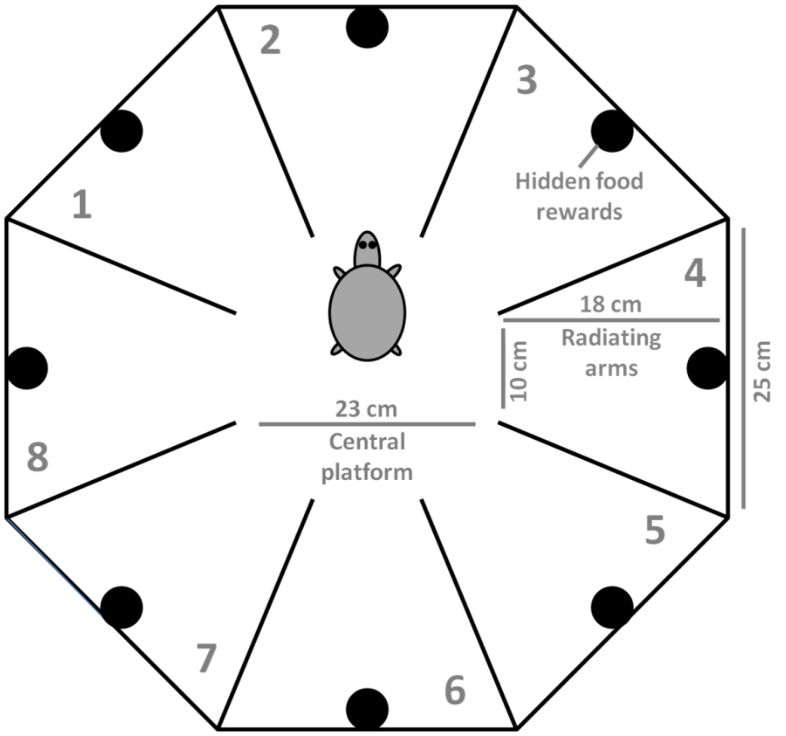

Figure 1: Radial-arm maze apparatus.

The maze was placed centrally in a 2.24 x 2.24 m room which was lit with two 25-W fluorescent tube lights. Each wall of the room contained a variety of visual stimuli that the tortoises could potentially use as cues during navigation. All the walls were white. One contained two sinks and soap dispensers, a large strip of beige newspaper ran across the entire wall, and there were two posters. A second contained a blue shelf, a lilac towel, a black, hanging plastic bag, two pictures, and a black door with a blue poster. The third white wall showed an array of many posters, a green, hanging plastic bag and an open doorway to an illuminated white-tiled wall and a black door. The fourth wall had an open doorway, leading to a dark room, a shelf containing several boxes, a yellow, hanging plastic bag, and a beige electric hand dryer.

Procedure

Experiment 1 was run over a period of 27 weeks between 7th September 2009 and 12th March 2010. The animals were tested five days a week, trials being given between 9.00 am and 5.00 pm.

Pre-training: To habituate them to the apparatus the animals were placed into the maze individually for 30 minutes with openly visible food items in each arm. Pre-training was considered complete when the animals had moved around and eaten readily for three sessions.

Training: Throughout the training phase each of the eight arms was baited with one hidden reward. Reward type varied across trials but within one trial all eight rewards were identical. At the start of each trial a tortoise was individually placed into the maze facing a randomly selected arm. The tortoise was allowed to move around the maze freely and to collect the food rewards from the different arms. Throughout each trial the experimenter observed and documented the tortoise's behaviour on a monitor from a room adjacent to the testing room. A video set-up above the maze provided a live feed of the animal's behaviour for this purpose. The order in which each animal entered the arms was recorded. Entry into an arm was counted when half the animal's body was inside the arm. Entering an arm that had not previously been visited was counted as a correct choice and a repeat visit within a trial was considered an error. The tortoises were run between one and five times per day. A trial was terminated when eight arms (regardless of whether the choices were to novel or previously visited arms) had been visited. Trials were terminated and rerun later if the animal failed to move for 15 min, or after 30 min (extended to 40 min if the animal was actively foraging) even though fewer than eight choices had been made. Behaviour observed on such incomplete trials was scored but not analysed further.

To ensure reliable observation of arm choice behaviour, inter-observer reliability was tested on 240 potential arm-choices made by three different tortoises over ten sessions each.

These reliability test trials were analysed both by the experimenter and by an additional observer who was blind to the hypothesis of the study. Inter-observer reliability was 100%.

All the animals, apart from Emily, received training until they had completed 120 trials; Emily's performance was successful and stable from early in training and she received only 40 trials. After completing this first phase of training, all animals received two different tests designed to examine what cues they were using to navigate around the maze.

Food odour test: This test examined whether the tortoises used the scent of the rewards to guide their selection of novel arms. Test sessions consisted of a training trial followed by a test trial. One to two test sessions were run per day. Test trials were identical to training trials except that only four arms were baited. Four test trials were run for each animal with the bait being presented in arms 1, 3, 5, and 7, and in arms 2, 4, 6, and 8 on alternate trials. Following this food odour test, the tortoises received retraining until they achieved eight successful trials before being undergoing the next test.

Scent trail test: This test was designed to examine whether the tortoises used scent trails as a cue to avoid a previously visited arm. The test trials resembled training trials in that all arms were baited but the maze floor was flooded with a 5 mm layer of warm water to disperse olfactory cues. The water remained in the maze throughout each scent trail test trial but was exchanged between trials. Each tortoise received eight test trials with a maximum of two trials per day.

Results

All four tortoises readily explored the maze during the pre-training phase and required only three to five trials to meet habituation criterion.

Training: In the course of a trial of 8 choices, the chance expectation is that 5.3 would be novel arm choices (Olton & Samuelson, 1976)**.** By the end of training all animals were performing above this chance level. One-sample t-tests comparing the mean number of novel arm-choices over the last 20 trials to the chance expectation (5.3 arms) showed that all four tortoises visited significantly more novel arms than could be expected on the basis of chance. For Molly the mean score was mean 6.0 ($t(19) = 2.772$, $p < .05$), for Esme, 5.9 ($t(19) = 2.676$, $p < .05$), for Quinn, 6.0 ($t(19) = 2.207$, $p < .05$), and for Emily it was 7.3 ($t(19) = 10.251$, $p < .001$).

Food odour test: Figure 2a shows, for each subject, the number of novel arms entered over all four test trials, distinguished according to whether the chosen arm was baited or not. It is evident that there was no tendency to preferentially choose arms that were baited. Chi-square tests showed no significant difference in the likelihood of a visit to a baited versus an unbaited arm for any of the tortoises, with Molly $\chi^2(1) = 0.39$, $p > .05$, Esme $\chi^2(1) = 0.18$, $p > .05$, Quinn $\chi^2(1) = 0.04$, $p > .05$, and Emily $\chi^2(1) = 0.04$, $p > .05$.

Scent trail avoidance test: Figure 2b shows, for each subject, the mean number of novel arms chosen over the 8 test trials in the flooded maze. Also shown, for comparison, is the mean number of novel arms chosen over the last eight trials of the training phase (i.e., in the dry maze). It is evident that flooding the maze had little effect on performance. Paired sample t-tests comparing the test performance to the performance during the last eight training trials found no significant differences for any tortoise: Molly, $t(7) = -1.16$, $p > .05$; Esme, $t(7) = 1.00$, $p > .05$; Quinn, $t(7) = 0.21$, $p > .05$; and Emily, $t(7) = -0.764$, $p > .05$.

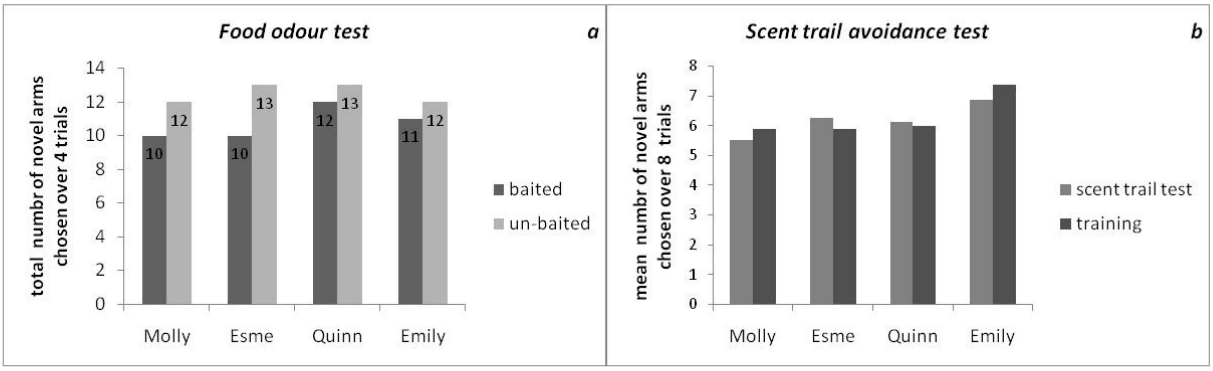

Figure 2: Performance of four red-footed tortoises on two olfactory tests. (a) Total number of visits to baited versus unbaited arms, (b) Mean number of novel arms chosen during the last eight training and the eight scent trail avoidance test trials

Turning behaviour: For each completed trial for each tortoise, a record was made of the exact pattern of turns made. This allowed us to score the number of turns between adjacent arms (one-arm turns) that occurred in succession in a given turning direction. Such a response strategy would constitute an efficient way of behaving in the radial maze. We also scored the number of two-arm, three-arm, and four-arm turns in a row in given direction for each trial. Figure 3 shows, for each subject, the mean number of turns of each type throughout successive four-trial blocks of the training phase. It is evident that Emily and Quinn readily developed a turn-by-one arm strategy. For Esme, there was a slight tendency for turns by two arms to predominate, whereas Molly showed no clear pattern.

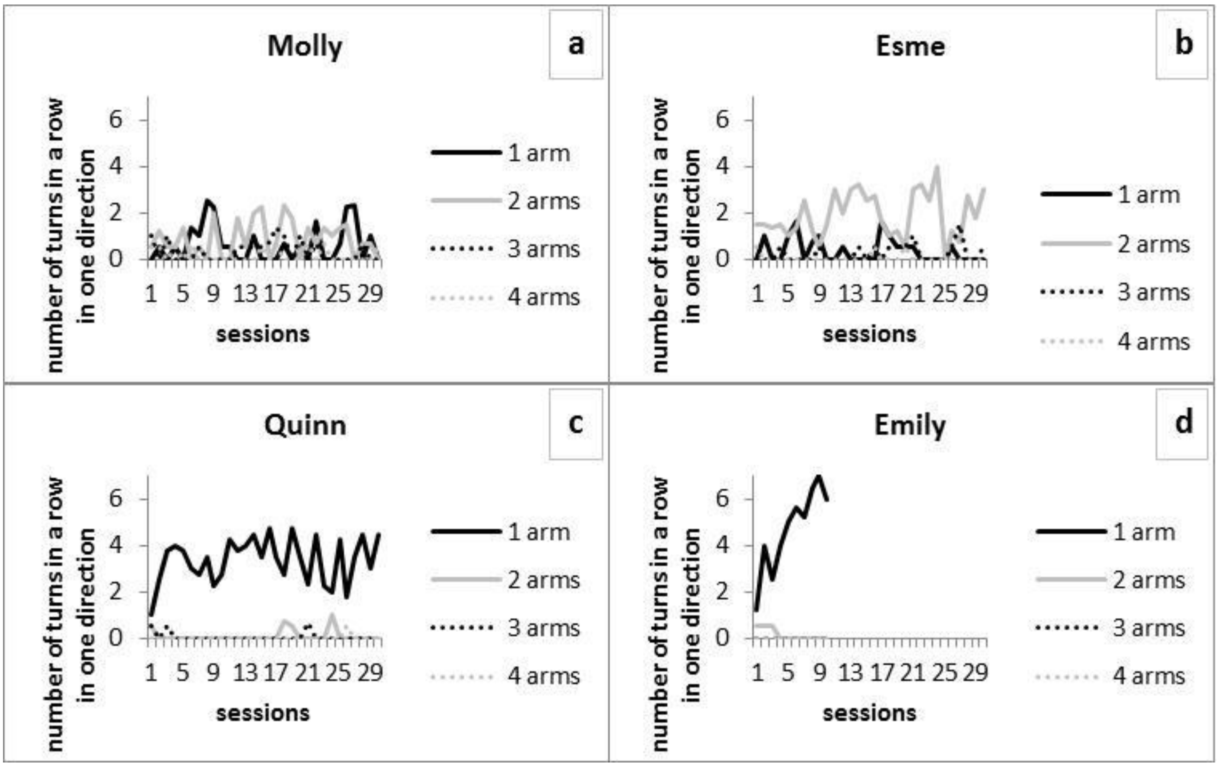

Figure 3: Turning behaviour during Experiment 1: the number of turns in a row in one direction for each turn type in blocks (sessions) of four trials.

For statistical analysis we concentrated on the performance shown on the first 20 and last 20 trials of training (Table 1). For each tortoise on each of these blocks we conducted a chi-square test looking at the proportional use of the four different turn types. The results of these tests are reported at the top of the column labelled SR in Table 1. In every case but one (that of Molly for the beginning of training) a significant effect was obtained, indicating that the various turn types were being used differentially. Analyses of the standardized residuals (SR) revealed which turn types contributed to the significant results of the chi-square analyses. SRs were calculated from the observed (O) and expected (E) values of each cell with the following formula: $SR = (O-E)/E^2$. SRs of above 1.96 indicated a significantly larger than expected contribution of the cell, SRs below -1.96 a significantly lower than expected contribution and SRs between 1.96 and -1.96 a non-significant contribution. This analysis confirmed that Quinn and Emily, showed a significantly enhanced tendency to perform one-arm turns and correspondingly, a reduced tendency to make other types of turns during both the first and the last 20 trials of training. Esme produced a larger-than-expected number of two arm turns both during the first and the last 20 trials. Molly showed no very clear pattern, although there was a significant tendency to favour two-arm turns at the end of the training.

Table 1: Turning behaviour of four red-footed tortoises based on chi-square calculations and analyses of the resulting standardized residuals (SR), SR = (Observed-Expected)/Expected^2, (* significant at .05 (SR > +1.96 or <-1.96)).

		Experiment 1				**Experiment 2**				**Experiment 3**				**Experiment 4**			
		First 20 trials		**Last 20 trials**		**First 20 trials**		**Last 20 trials**		**First 20 trials**		**Last 20 trials**		**First 20 trials**		**Last 20 trials**	
	Turn types	SR	Turns Used	SR	Turns used	SR	Turns used	SR	Turns used	SR	Turns used	SR	Turns used	SR	Turns used	SR	Turns used
Molly		$\chi^2(3) = 5.06$ p >.05		$\chi^2(3) = 11.67$ p < .05		$\chi^2(3) = 5.06$ p < .001		$\chi^2(3) = 126.10$ p < .001		$\chi^2(3) = 86.11$ p < .001		$\chi^2(3) = 41.68$ p < .001		$\chi^2(3) = 59.85$ p < .001		$\chi^2(3) = 15.68$ p < .001	
	1	-0.86		-0.69		4.52	More	9.62*	More	7.77*	More	4.99*	More	6,10	More	1,82	
	2	1.89		2.76*	More	0.52		-2.15*	Less	-0.84		-2.29*	Less	0,55		1,50	
	3	-0.17		-1.84		-2.06*	Less	-3.06*	Less	-2.75*	Less	0.57		-3,05	Less	-0,06	
	4	-0.86		-0.31		-2.92*	Less	-4.42*	Less	-4.18*	Less	-3.33*	Less	-3,61	Less	-3,19	Less
Esme		$\chi^2(3) = 41.93$ p < .001		$\chi^2(3) = 66.32$ p < .001		$\chi^2(3) = 39.53$ p < .001		$\chi^2(3) = 109.60$ p < .001		$\chi^2(3) = 106.63$ p < .001		$\chi^2(3) = 116.71$ p < .001		$\chi^2(3) = 149.23$ p < .001		$\chi^2(3) = 142.53$ p < .001	
	1	0.06		-3.54*	Less	-2.75*	Less	-2.32*	Less	-2.53*	Less	-2.94*	Less	-5.92*	Less	-5.58*	Less
	2	5.23*	More	6.93*	More	5.32*	More	9.04*	More	8.89*	More	9.35*	More	3.72*	More	3.38*	More
	3	-2.68*	Less	-1.84		-0.66		-3.36*	Less	-2.29*	Less	-3.21*	Less	-5.07*	Less	-5.41*	Less
	4	-2.68*	Less	-1.56		-1.85		-3.36*	Less	-4.02*	Less	-3.21*	Less	-5.92*	Less	-5.92*	Less
Quinn		$\chi^2(3) = 147.50$ p < .001		$\chi^2(3) = 197.45$ p < .001		$\chi^2(3) = 156.00$ p < .001		$\chi^2(3) = 245.37$ p < .001		$\chi^2(3) = 233.39$ p < .001		$\chi^2(3) = 305.30$ p < .001		$\chi^2(3) = 136.80$ p < .001		$\chi^2(3) = 126.01$ p < .001	
	1	10.50*	More	12.14*	More	10.82*	More	13.54*	More	13.20*	More	15.11*	More	10,07	More	9,47	More
	2	-3.50*	Less	-4.22*	Less	-3.61*	Less	-4.67*	Less	-4.12*	Less	-4.79*	Less	-2,32	Less	-1,27	
	3	-3.00*	Less	-4.22*	Less	-3.61*	Less	-4.24*	Less	-4.56*	Less	-5.18*	Less	-3,87	Less	-3,68	Less
	4	-4.00*	Less	-3.74*	Less	-3.61*	Less	-4.67*	Less	-4.56*	Less	-5.18*	Less	-3,87	Less	-4,56	Less
Emily		$\chi^2(3) = 163.17$ p < .001		$\chi^2(3) = 360.00$ p < .001		$\chi^2(3) = 341.27$ p < .001		$\chi^2(3) = 211.50$ p < .001		$\chi^2(3) = 294.00$ p < .001		$\chi^2(3) = 369.00$ p < .001		$\chi^2(3) = 280.92$ p < .001		$\chi^2(3) = 290.31$ p < .001	
	1	11.04*	More	16.43*	More	15.97*	More	12.56*	More	14.85*	More	16.61*	More	14,51	More	14,75	More
	2	-2.70*	Less	-5.48*	Less	-5.46*	Less	-3.09*	Less	-4.95*	Less	-5.55*	Less	-4,71	Less	-4,65	Less
	3	-4.15*	Less	-5.48*	Less	-5.09*	Less	-4.51*	Less	-4.95*	Less	-5.55*	Less	-4,71	Less	-5,05	Less
	4	-4.15*	Less	-5.48*	Less	-5.46*	Less	-4.92*	Less	-4.95*	Less	-5.55*	Less	-5,10	Less	-5,05	Less

Differences between the use of each turn type during the first and last twenty trials were analysed by repeated measures t-tests. They revealed that Molly significantly reduced the number of three-arm-turns (t(19) = 2.540, p < .05) and Esme significantly reduced the number of one-arm-turns (t(19) = 4.931, p < .05) while increasing the number of four-arm-turns (t(19) = -2.162, p < .05). Both Quinn and Emily significantly increased the number of one-arm-turns (Quinn: t(19) = -2.266, p < .05, Emily: t(19) = -4.094, p < .05) and significantly decreased the number of two-arm-turns (Quinn: t(19) = 2.668, p < .05, Emily: t(19) = 3.559, p < .05).

Discussion

The results show that all four red-footed tortoises were able to perform in the eight-arm radial maze at a level significantly better than chance. This suggests that mastering a radial-arm maze task lies within the general capabilities of red-footed tortoises and is not a peculiarity of the one tortoise previously tested by Wilkinson et al. (2007, 2009). Over the last 20 trials of training all had scores (mean number of novel arms entered in 8 choices) that differed significantly from the chance level (the poorest performing subject had a score of 5.9; the best, of 7.3).The tortoises were thus slightly less proficient than rats in this task (Olton & Samuelson, 1976), but they performed at a level similar to that shown by pigeons (Bond et al., 1981), by the jewelled lizard studied by Mueller et al., and the red-footed tortoise tested previously by Wilkinson et al. (2007, 2009).

Maze orientation did not appear to be based on odour cues emanating from the rewards, as the tortoises did not choose baited arms more often than un-baited ones, nor is performance likely to depend on scent trails left by the tortoises themselves, as no disruption in behaviour was observed during the scent trail avoidance test when scent cues were dispersed by water. Even if flooding the maze might not have entirely removed all scent cues, the tortoises' movement around the maze would have dispersed them in a manner sufficient to introduce a clear decrease in performance had the animals relied on scent trails to any major degree when solving the task. It is further unlikely that the animals were leaving scent cues in the form of defecation or urination in the maze as these behaviours were only infrequently observed and marks removed immediately after the trial during which they occurred. In summary, the tortoises' behaviour regarding navigation based on scent is in line with the behaviour found in rats (Olton & Collison, 1979).

In other respects the behaviour of the tortoises was very different from that shown by rats. All of the tortoises performed at a high level from the beginning of training, and only one showed a significant improvement over time. For two of the subjects their successful performance was the consequence of the rapid development of a turn-by-one arm strategy. Turning by one arm is a response strategy that will obviously lead to a high success rate (Dale & Innis, 1986). In so far as the other two animals developed a response strategy, it tended to involve turning by two arms. Clearly, a continuous sequence of two-arm turns in an eight-arm radial maze can lead to no more than four correct choices. The consistent, above-chance, level of maze performance shown by these subjects thus indicates that additional mechanisms must have been operating. This could have involved some reliance on the extra-maze cues, or it may have involved additional response rules (such as performing a different turn type as soon as an empty aisle is encountered or after every three choices). Evidence favouring the former interpretation comes from the behaviour shown by the tortoise tested by Wilkinson et al. (2007, 2009). When extra-maze cues were not available it used one-arm turns, whereas two-arm turns prevailed when such cues were numerous. One possibility, then, is that when navigation is largely based on the extra-maze cues, the two-arm turning behaviour predominates simply because such turns are considerably easier than turning by one arm (Yoerg & Kamil, 1982), particularly for a physically inflexible animal like a tortoise. This suggestion is investigated in the next experiment.

Experiment 2: *What determines navigation mechanisms used by the red-footed tortoise?*

Introduction

In Experiment 1, the tortoises with a preference for one-arm turns were the two smaller ones, whose neck lengths are approximately 2 cm shorter than those of the two larger tortoises. Thus, the smaller tortoises' heads were lower in the maze which might have prevented them from detecting visual cues from the room that were more readily available to the larger tortoises. This could have resulted in the smaller tortoises relying on response strategy whereas the larger tortoises were able to see the room cues clearly and to employ a memory strategy accompanied by the easier turn type.

To test the hypothesis the differential visual access to the extra-maze has resulted in the different navigations methods chosen by the tortoises we designed a second experiment in which the maze walls were raised for the larger tortoises and lowered for the smaller tortoises. These amendments should provide better visual access to cues for the smaller tortoises and reduce the cue information accessible to the larger tortoises. The question of interest was whether the latter would then resort to a turn-by-one-arm strategy whereas the former would abandon this strategy.

Methods

The experiment was run over a period of 10 weeks between 22nd February 2010 and 05th May 2010. The animals were tested five days a week between 9 am and 5 pm until they had received 80 trials each. The same four juvenile red-footed tortoises that participated in Experiment 1 took part in this experiment. No changes were made to training procedure.

Apparatus

The apparatus consisted of two eight-arm radial mazes of identical size and design to that used in Experiment 1. The only difference was in the height of the maze walls. The outer walls of the large tortoises' maze were raised by 5 cm to a total height of 19 cm by attaching black cardboard to the outside of the maze. The floor of the small tortoises' maze was raised by inserting a 5-cm thick layer of firm rubber foam covering the entire maze floor. The rubber foam was covered with the same grip-ensuring rubber lining as before so that there was no apparent difference from the previous maze floor. The resultant wall height of this maze was 9 cm. The maze position and the testing room were kept identical.

Results

Training: Maze performance compared to chance (mean 5.3 arms) during the first and last 20 trials of radial-arm maze training was analysed using one-sample t-tests. These showed above chance performance for all four tortoises, Molly (first 20 trials: mean 6.1, t(19) = 4,198, $p < .001$; last 20 trials: mean 6.2, t(19) = 4,500, $p < .001$), Esme (first 20 trials: mean 5.8, t(19) = 2,500, $p < .05$; last 20 trials: mean 6.2, t(19) = 4,230, $p < .001$), Quinn (first 20 trials: mean 6.4, t(19) = 7,998, $p < .001$; last 20 trials: mean 6.3, t(19) = 3,963, $p < .001$), and Emily (first 20 trials: mean 7.5, t(19) = 14,009, $p < .001$; last 20 trials: mean 6.6, t(19) = 5,086, $p < .001$). A comparison of the tortoises' performance during the first and the last 20 trials showed a significant decrease only for Emily t(19) = 2.93, $p < .05$.

Turning behaviour: For each completed trial for each tortoise, a record was kept of the exact pattern of turns made and the number of one-arm, two-arm, three-arm and four-arm turns in a row in one direction was counted. The tortoises' turning behaviour was analysed as described in Experiment 1. Figure 4 shows, for each subject, the mean number of turns of each type throughout successive four-trial blocks of the training phase. It is evident that Emily and Quinn maintained a turn-by-one arm strategy and Esme appeared to develop a strong preference for two-arm-turns. When presented with restricted room cues Molly developed a clear preference for one-arm-turns.

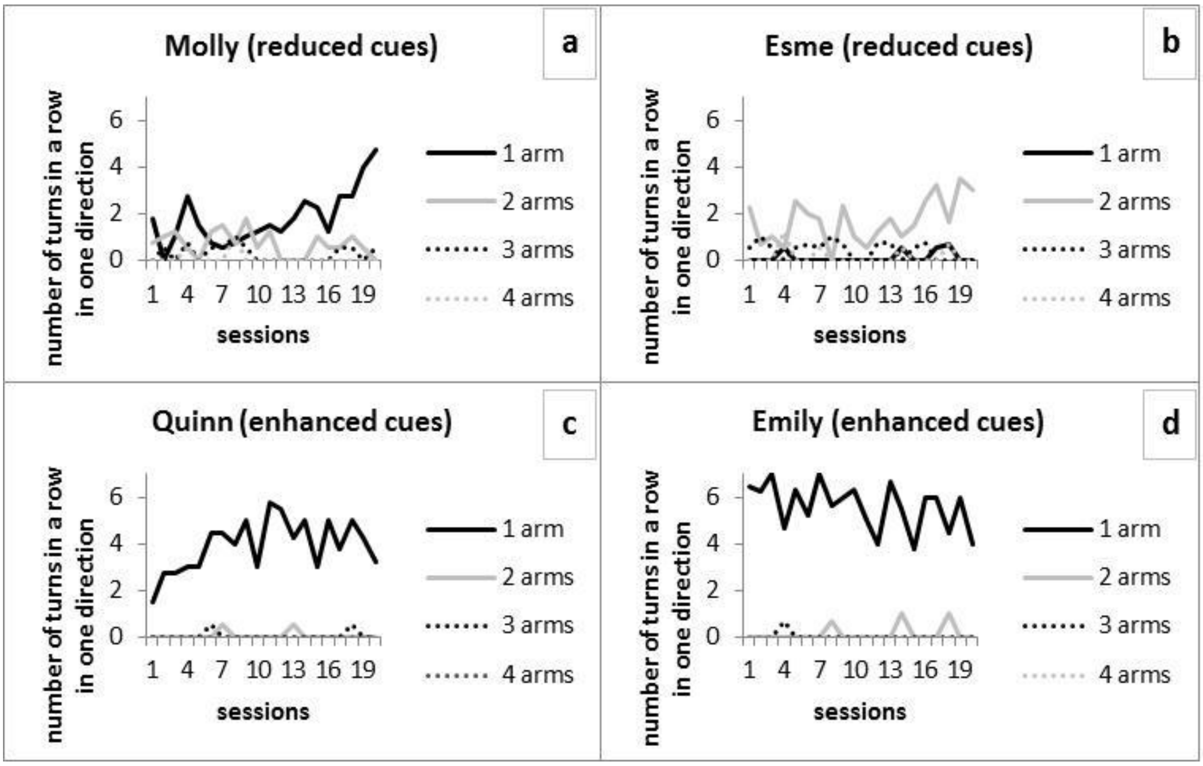

Figure 4: Turning behaviour of four red-footed tortoises during experiment 2 with reduced and enhanced cues, respectively: the number of turns in a row in one direction for each turn type in sessions of four trials.

Chi-square tests revealed a highly significant difference in number of turn types for the first and last 20 trials for all four tortoises (*Table 1*). Analyses of the SRs showed that Molly, Quinn and Emily used significantly more one-arm turns than all other turn types, whereas Esme used significantly more two-arm turns during both the first and last 20 trials (table 1). Differences between the turning behaviour of the first and last twenty trials analysed by repeated measures t-tests revealed that Molly significantly increased the number of one-arm-turns (t(19) = -2.861, $p < .05$) and Esme significantly increased the number of two-arm-turns (t(19) = -2.430, $p < .05$). Quinn significantly increased the number of one-arm-turns (t(19) = -4.003, $p < .05$) while Emily significantly decreased the number of one-arm-turns (t(19) = 3.187, $p < .05$) and increased the number of two-arm-turns (t(19) = -2.131, $p < .05$).

Discussion

All four tortoises maintained above-chance performance in the changed conditions of this experiment. Analyses of their turning behaviour revealed that Molly changed her choice behaviour. When visual access to the room cues was impaired by raising the maze walls by 5 cm she started using a response strategy of sequentially turning into adjoining arms. This change in behaviour suggests that Molly might initially have been using a strategy that relied on visual cues but that when access to these cues was reduced she adapted by adopting a response strategy of one-arm turns. This is demonstrated by the significant increase in the number of one-arm turns towards the end of the experiment. Her behaviour corresponds with that of the red-footed tortoise tested by Wilkinson et al. (2009). The second large tortoise, Esme, did not abandon her preferred two-arm turn strategy; indeed she showed an increased tendency to make such turns.

The other two tortoises, the smaller animals, Quinn and Emily, did not show a strategy change in response to the increased visual access to the room cues resulting from the lowering of the maze walls. Both maintained high levels of response stereotypy in the form of one-arm turns (although Emily's overall performance and continuous turning behaviour decreased somewhat towards the end of the experiment as she increased the use of two-arm- and reduced the use of one-arm turns). In neither animal, therefore, was there evidence of a shift to a strategy that made use of the extramaze cues. It seems, therefore, that a response-based strategy, once established, is difficult to change. This matter was investigated further in the next experiment.

Experiment 3: Can existing response stereotypy be modified?

Introduction

We had anticipated that Quinn and Emily might abandon their response-based strategy when, in Experiment 2, the extramaze cues were made more accessible. That they did not do so may indicate only that our technique for increasing the availability of these cues was not powerful enough. Accordingly, in this experiment we continued training them in the modified maze, but enhanced the properties of the room cues. We also continued training with Molly and Esme in the presence of these cues, but with the maze used in Experiment 1. The question of interest in this case was whether Molly, who had changed to a turn-by-one-arm strategy in Experiment 2, would revert to her previous pattern of behaviour.

Methods

The experiment was run over a period of 13 weeks between 20th May 2010 and 23th August 2010. The animals were tested five days a week between 9 am and 5 pm until they had reached 80 trials each. The same four juvenile red-footed tortoises that participated in Experiments 1 and 2 took part in this experiment. No changes were made to the training procedure.

Apparatus

The apparatus consisted of the radial mazes that were used in Experiment 2. They differed only in that the outer walls of the large tortoises' maze were reduced back to their original height of 14 cm. The floor of the small tortoises' maze remained raised with the wall height of 9 cm. However, the cue environment in the testing room was changed. Although room cues such as doors, sinks, and posters remained in place, additional cues were moved closer to the maze. Specifically, two additional shelves stacked with a variety of colourful 3-D cues were placed at a distance of 25 from cm each side of the maze.

Results

Training: One sample t-tests analysing maze performance compared to chance (mean 5.3 arms) during the first and last 20 trials of radial-arm maze training showed that the mean number of arm visits was significantly higher than expect based on chance for all four tortoises, Molly (first 20 trials: mean 6.3, t(19) = 6.104, p < .001; last 20 trials: mean 6.0, t(19) = 3.647, p < .05), Esme (first 20 trials: mean 6.3, t(19) =4.396, p < .001; last 20 trials: mean 6.2, t(19) =4.344, p < .001), Quinn (first 20 trials: mean 6.2, t(19) =2.669, p < .05; last 20 trials: mean 6.6, t(19) = 5.557, p < .001), and Emily (first 20 trials: mean 6.9, t(19) = 7.015, p < .001; last 20 trials: mean 7.3, t(19) = 8.675, p < .001). A comparison of the tortoises' performance during the first and the last 20 trials did not show a difference in overall performance.

Turning behaviour: For each completed trial for each tortoise, a record was kept of the exact pattern of turns made and the number of one-arm, two-arm, three-arm and four-arm turns in a row in one direction was counted. The tortoises' turning behaviour was analysed as described in Experiment 1. Figure 5 shows, for each subject, the mean number of turns of each type throughout successive four-trial blocks of the training phase. Emily and Quinn maintained a turn-by-one arm strategy and Esme, apparently, maintained a turn-by-two arm strategy. Molly, however, returned to showing no obvious pattern of preferred arm types.

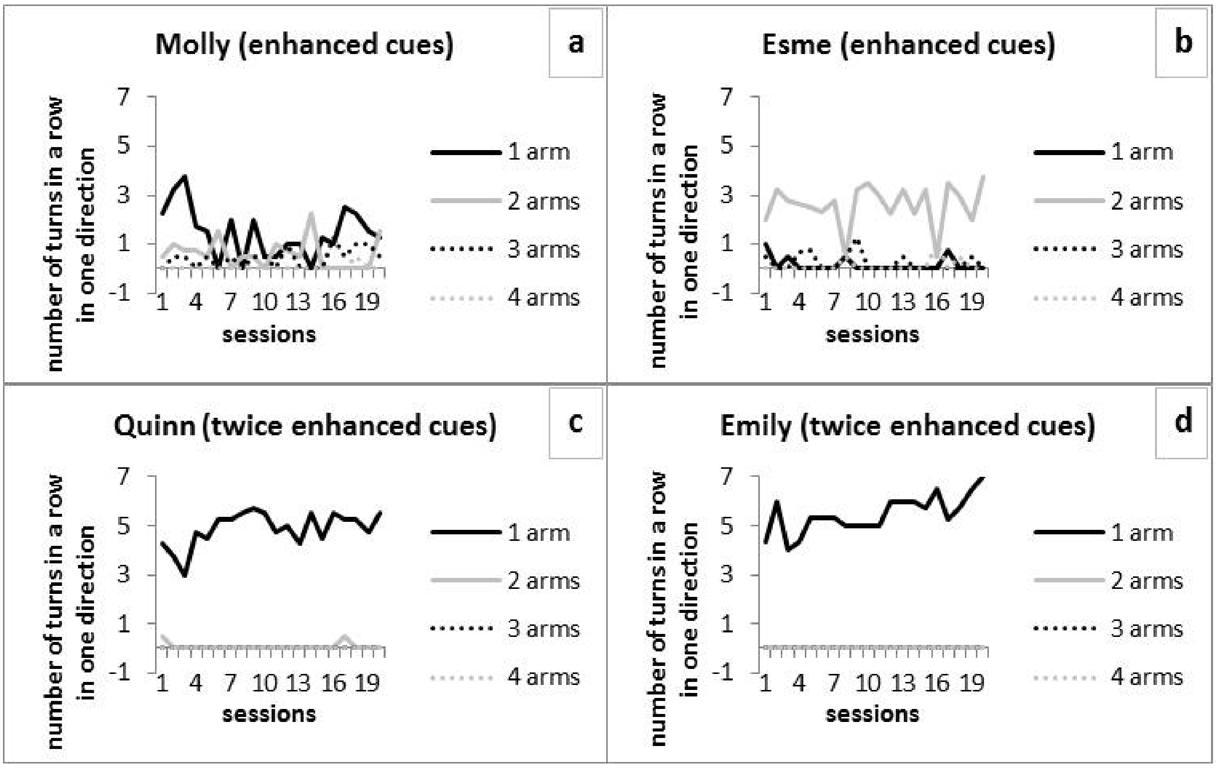

Figure 5: Turning behaviour of four red-footed tortoises during experiment 3 with enhanced cues and twice enhanced cues (additional room cues and low maze walls), respectively: the number of turns in a row in one direction for each turn-type in sessions of four trials.

Chi-square tests revealed a highly significant difference in number of turn types for the first and last 20 trials for all four tortoises (*Table 1*). Analyses of the SRs showed that Molly, Quinn and Emily used significantly more one-arm turns and Esme significantly more two-arm turns than expected on the basis of chance (*Table 1*). Differences between the turning behaviour of the first and last twenty trials analysed by repeated measures t-tests revealed no significant changes for either Molly or Esme. Quinn significantly reduced the number of two-arm-turns (t(19) = 2.463, p < .05) while Emily significantly increased the number of one-arm-turns (t(19) = -3.584, p < .05) and decreased the number of two-arm-turns (t(19) = -4.067, p < .05).

Discussion

The attempt to enhance the effectiveness of extramaze cues had no effect on the behaviour shown by Emily and Quinn who continued with the turn-by-one-arm strategy. This suggests that for them visibility and availability of visual cues might not play the deciding role in their navigational mechanism, or at least, that when it has been established initially (and remains successful), a response-based strategy is relatively impervious to changes in the environmental conditions.

Esme, too, continued with the strategy (in her case, turn by two arms) that was evident at the end of Experiment 2. Only Molly showed a degree of sensitivity to the changed conditions. Although there was some tendency for the turn-by-one strategy to dominate over others, her behaviour in this study reverted to that shown in Experiment 1. It seems unlikely that her slight preference for one-arm turns constituted the primary source of her successful overall performance. Combinations of memory strategies with stereotyped response strategies have repeatedly been observed in rats (Bond et al., 1981; Dale, 1981; Dale & Innis, 1986). But to demonstrate that external cue use is an important factor in Molly's navigation, it is necessary to test her without access to any visual cues. We took up this issue in Experiment 4

Experiment 4: Are the tortoises using response strategies or memory strategies?

Introduction

In Experiment 4 the role played by extramaze cues was examined by testing the while the maze was entirely surrounded by a black curtain, thus eliminating such cues. Animals whose behaviour is determined primarily by response-based strategies (i.e., Quinn and Emily) might be expected to continue to perform well in these conditions. A reduction in level of performance might be taken to indicate that their primary, response-based, strategy was supplemented by a secondary strategy requiring access to visual cues (although it would also be consistent with generalization decrement brought about by the general change in conditions).

Potentially more informative is the effect that this manipulation might have on the behaviour of Molly and Esme, at the end of Experiment 3, neither of these animals had response strategies fully capable of generating the overall performance they showed. To the extent that their behaviour depended on the use of external cues, performance levels should drop and use of other strategies, such as stereotyped responding, might well increase.

Methods

The experiment was run with Esme over a period of 9 weeks between 8th September 2010 and 11th October 2010 and with Molly, Quinn, and Emily over a period of 8 weeks from 06th December 2010 to 28th January. Because of the break between Experiments 3 and 4 for Molly, Quinn and Emily these three tortoises received 8 retraining trials to regain previous performance levels. The tortoises were tested five days a week between 9 am and 5 pm until they had reached 40 trials each.

Apparatus

The apparatus was the eight-arm radial maze as described for Experiment 1. The maze was entirely surrounded by a black curtain. An additional light was attached to the ceiling above the maze to compensate for the reduction in illumination produced by introduction of the curtain.

Procedure

No changes were made to the training procedure. After the 40 trials of the training phase the animals were given the two odour tests (the food odour and the scent trail test) exactly as described for Experiment 1. Finally, the animals received a further food odour test consisting of four trials (as before) but in which the bait was alternately presented in arms 1, 2, 5, and 6, and in arms 3, 4, 7, and 8 (the test in which alternate arms are baited allows a possible confound when animals have adopted a turn-by-two arm strategy).

Results

Training: One sample t-tests analysing maze performance compared to chance (mean 5.3 arms) during the first and last 20 trials of radial-arm maze training showed that the mean number of arm visits was significantly higher than expect based on chance of all four tortoises, with Molly (first 20 trials: mean 5.9, t(19) = 3.667, $p < .05$; last 20 trials: mean 5.9, t(19) = 3.736, $p < .001$), Esme (first 20 trials: mean 6.2, t(19) = 4.500, $p < .001$; last 20 trials: mean 6.2, t(19) = 3.847, $p < .001$), Quinn (first 20 trials: mean 6.2, t(19) = 3.141, $p < .05$; last 20 trials: mean 6.4, t(19) = 4.491, $p < .001$), and Emily (first 20 trials: mean 6.6; t(19) = 4.895, $p < .001$; last 20 trials: mean 7.0, t(19) = 7.813, $p < .001$).

Turning behaviour: For each completed trial for each tortoise, a record was kept of the exact pattern of turns made and the number of one-arm, two-arm, three-arm and four-arm turns in a row in one direction was counted. The tortoises' turning behaviour was analysed as described in Experiment 1. Figure 6 shows, for each subject, the mean number of turns of each type throughout successive four-trial blocks of the training phase. Given the complete obstruction of external cues Emily, Quinn and Esme maintained their previously developed strategies of turning by-one arm and by-two arms while Molly showed no clear pattern.

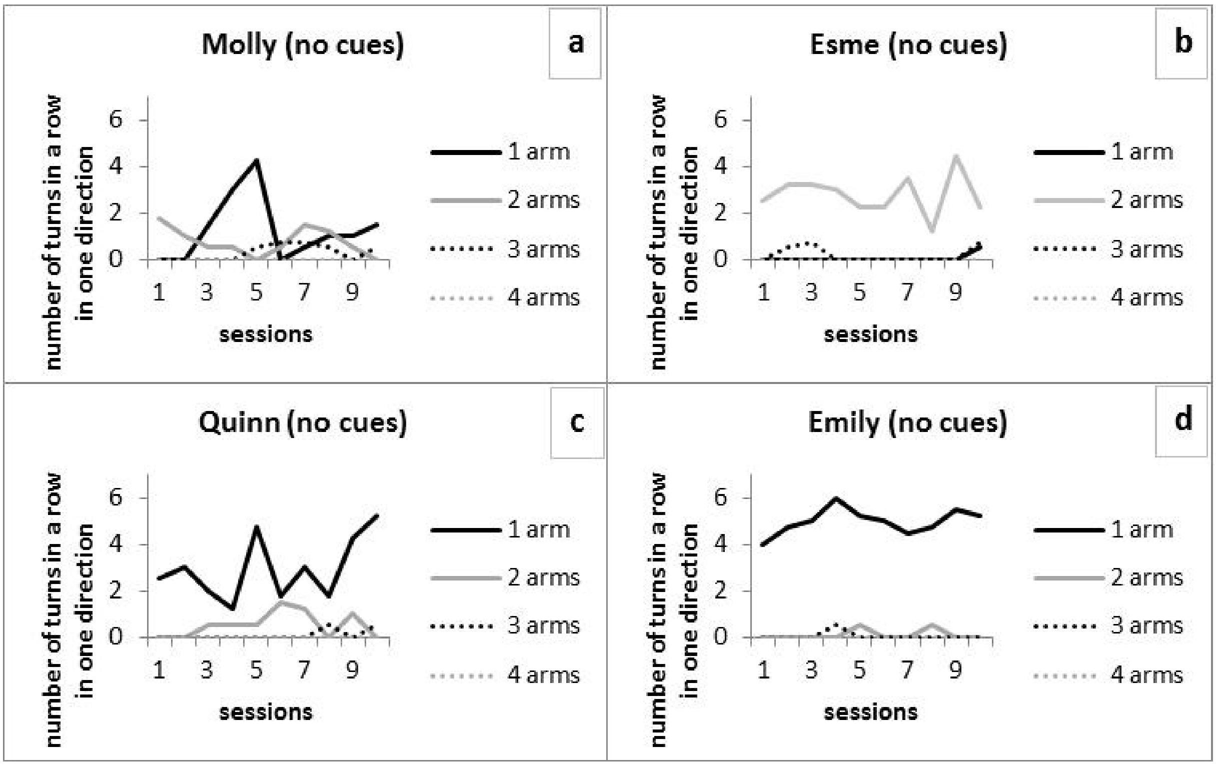

Figure 6: Turning behaviour of four red-footed tortoises during experiment 4 with no cues visible: the number of turns in a row in one direction for each turn type in sessions of four trials.

Chi-square tests revealed a highly significant difference in number of turn types for the first and last 20 trials for all four tortoises (*Table 1*). Analyses of the SRs showed that Esme used significantly more two-arm turns than expected on the basis of chance while Quinn and Emily used significantly more one arm turns than any other turn types during both the first and the last 20 trials. Molly used significantly more one arm turns during the first 20 trials but showed no above-chance turn-use during the last 20 trials (*Table 1*). Differences between the turning behaviour of the first and last twenty trials analysed by repeated measures t-tests revealed that Molly significantly reduced the number of one-arm-turns ($t(19) = 2.372$, $p < .05$) and increased the number of two-arm-turns ($t(19) = -2.483$, $p < .05$).

Food odour test: Chi-square tests showed no significant difference for either tortoise (Figure 7*a*) for neither the first (Molly: $\chi^2(1) = 2.33$, $p > .05$; Esme: $\chi^2(1) = 1.64$, $p > .05$; Quinn: $\chi^2(1) = 0.00$, $p > .05$; Emily: $\chi^2(1) = 0.04$, $p > .05$) nor the second round of testing

(Molly: $\chi^2(1) = 3.52$, $p > .05$; Esme: $\chi^2(1) = 0.00$, $p > .05$; Quinn: $\chi^2(1) = 0.00$, $p > .05$; Emily: $\chi^2(1) = 0.04$, $p > .05$). Both test rounds combined revealed that Molly chose the baited arms significantly more often than the un-baited arms ($\chi^2(1) = 5.82$, $p < .05$), while no significant difference was found for the other three tortoises (Esme: $\chi^2(1) = 0.82$, $p > .05$; Quinn: $\chi^2(1) = 0.00$, $p > .05$; Emily: $\chi^2(1) = 0.07$, $p > .05$).

Scent trail avoidance test: Paired sample t-tests comparing test performance to the performance during the last eight training trials found no significant differences for any tortoise with Molly $t(7) = 0.00$, $p > .05$, Esme $t(7) = -1.18$, $p > .05$, Quinn $t(7) = -1.51$, $p > .05$, and Emily $t(7) = -0.24$, $p > .05$ (*Figure 7b*).

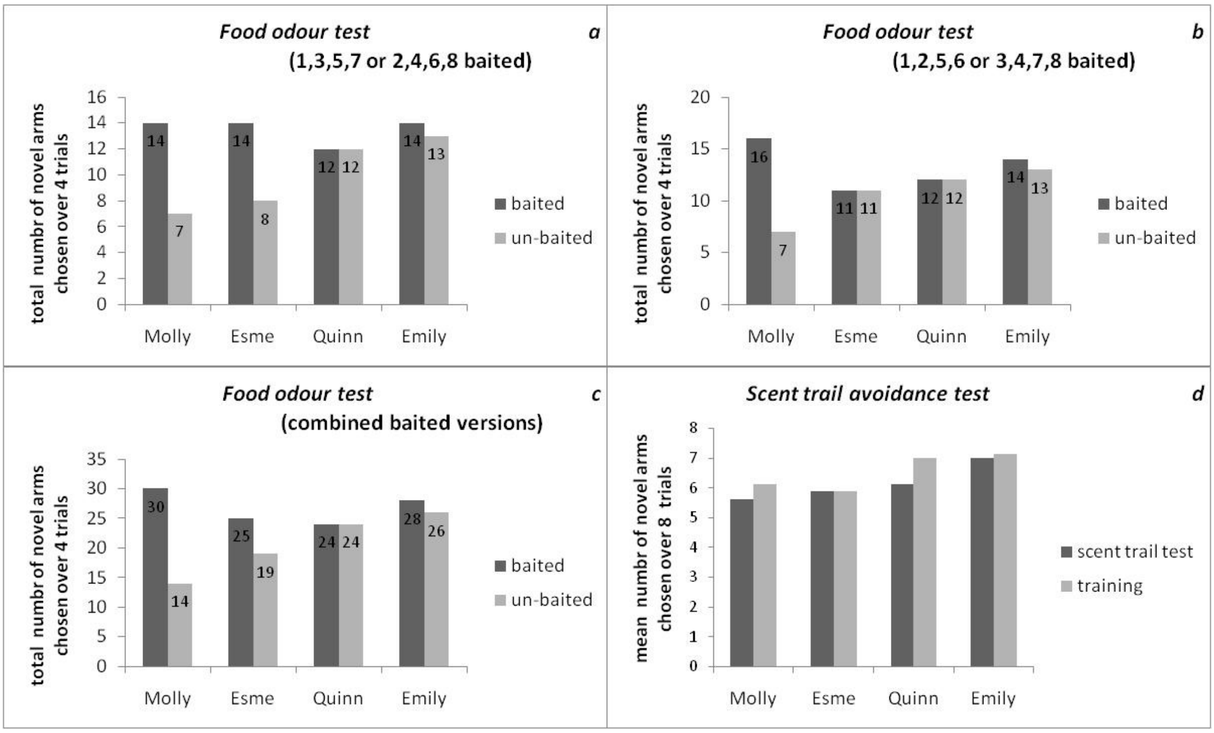

Figure 7: Performance of four red-footed tortoises on two olfactory tests. Panels a-c show the total number of visits to baited versus un-baited arms in the first test round, the second test round and the test rounds combined. Panel d shows the mean number of novel arms chosen during the last eight training and the eight scent trail avoidance test trials.

Discussion

In the no-cue condition the maze performance of all four tortoises remained above chance. Quinn and Emily’s turn-by-one arm performance remained high and Esme maintained a significant preference for two-arm turns. That no changes in overall performance or turning behaviour appeared when all visual cues were removed strongly indicates that the response stereotypy displayed by Esme, Quinn and Emily is likely to be a primary mechanism based on a response strategy rather than a secondary mechanism accompanying a memory strategy.

A change of behaviour following the removal of all visual cues was observed in Molly, in that her tendency to (slightly) prefer a turn-by-one arm strategy, evident at the end of Experiment 3, was no longer shown. She did not preferentially choose any turn-type but seemed to have developed a strategy based on the smell of the reward that enabled her to keep performance above chance level. The reduction in turn-by-one-arm behaviour in response to the total loss of visual cues is in contrast to the increase in this behaviour that was shown in response to a partial obstruction of visual stimuli in Experiment 2. Though not conclusive, this pattern of results may indicate that Molly was using the response strategy as a secondary method to facilitate memory-based navigation. It is therefore possible that she used visual, cue-related navigation strategies during the first three experiments. When no extramaze cues were available during Experiment 4 Molly may have started to use olfactory cues from the reward to solve the task. When cue availability was diminished in Experiment 2 this potential cue-based strategy was not abandoned but, given the increased difficulty of the task, was supplemented by an increase in turn-by-one-arm behaviour. This technique for reducing task demands and memory load has also been observed in rats (Dale & Innis, 1986). There is no obvious reason why Molly should have behaved differently from the other three subjects (all of whom showed a tendency to rely on stereotyped responding) but it is noteworthy that Molly was the only tortoise to show a learning curve during Experiment 1 which suggests that she applied a different approach to the other three tortoises from the beginning of the test series.

General discussion

The results of these experiments indicate that efficient eight-arm radial maze navigation is within the general capabilities of red-footed tortoises. Further, their performance, although slightly inferior to that observed in rats (Olton & Samuleson, 1976), appeared to be similar to that of pigeons (Bond et al,, 1981), of another red-footed tortoise (Wilkinson et al., 2009), and a jewelled lizard (Mueller et al, submitted). Analyses of the tortoises' choice behaviour revealed a preference for response strategies for maze navigation in three of the four animals tested. Thus tortoises, like fish (Hughes & Blight, 1999), appear to have a slightly greater preference for the use of primary response strategies in comparison with pigeons (Bond et al., 1981) and rats (Dale & Innis, 1986), which tend to prefer memory strategies accompanied by secondary response stereotypy. The source of this difference between the behaviour of the tortoises and that of birds and mammals may lie in a difference in error costs. Yoerg and Kamil (1982) have tested rats in mazes of different sizes. They found that increasing the size of the central platform, and with it the cost of errors, increases the use of response stereotypy. This is possibly because response stereotypy, as a primary mechanism, is highly successful in a stable experimental setting. When comparing the ease and speed of moving around the maze rats are likely to be the fastest followed by pigeons, and tortoises are expected to be slowest. Assuming that greater walking ease and speed correspond with lower error cost and a low rate of response strategy one might expect rats to show the least reliance on response strategies, and tortoises the most, with pigeons somewhere in the middle. This is exactly the picture that is found. Future studies could attempt to vary the error cost for the three different species systematically by, for example, adjusting the distance between the arms or the ease of walking in the maze, to test for changes in mechanism preferences.

The studies by Wilkinson et al, (2007, 2009) and the results of Molly in the present study show that at least some tortoises are able to use a memory strategy to successfully navigate in a radial arm maze and that they do possess a certain degree of flexibility in respect to navigation mechanisms. It is possible that this sort of behaviour may be characteristic of the natural foraging behaviour of tortoises – to rely on a stereotyped response strategies could be disadvantageous of in the wild where circumstances are likely to change frequently. Reliance on a response strategy could be a consequence of the experimental setting in which conditions are highly stable, and such strategies lead to high levels of success while providing the shortest routes and requiring low levels of working memory involvement. This hypothesis could be tested by increasing the level of variability within the experimental setup. Regular variations might encourage the use of memory strategies and demonstrate a more general degree of flexibility in the behaviour of the red-footed tortoise.

In conclusion, the radial maze behaviour of red-footed tortoises shows strong parallels to that of mammals and birds, in that both response stereotypy and memory strategies can be used. However, in the experimental setup used here, tortoises appear to have a stronger preference for the primary use of response stereotypy than is seen the other amniotes. It is possible that part of this difference can be explained by the cost of errors when navigating the maze.

Acknowledgements

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Chapter 4: Response-stereotypy in the jewelled lizard (*Timon lepidus*) in a radial-arm maze

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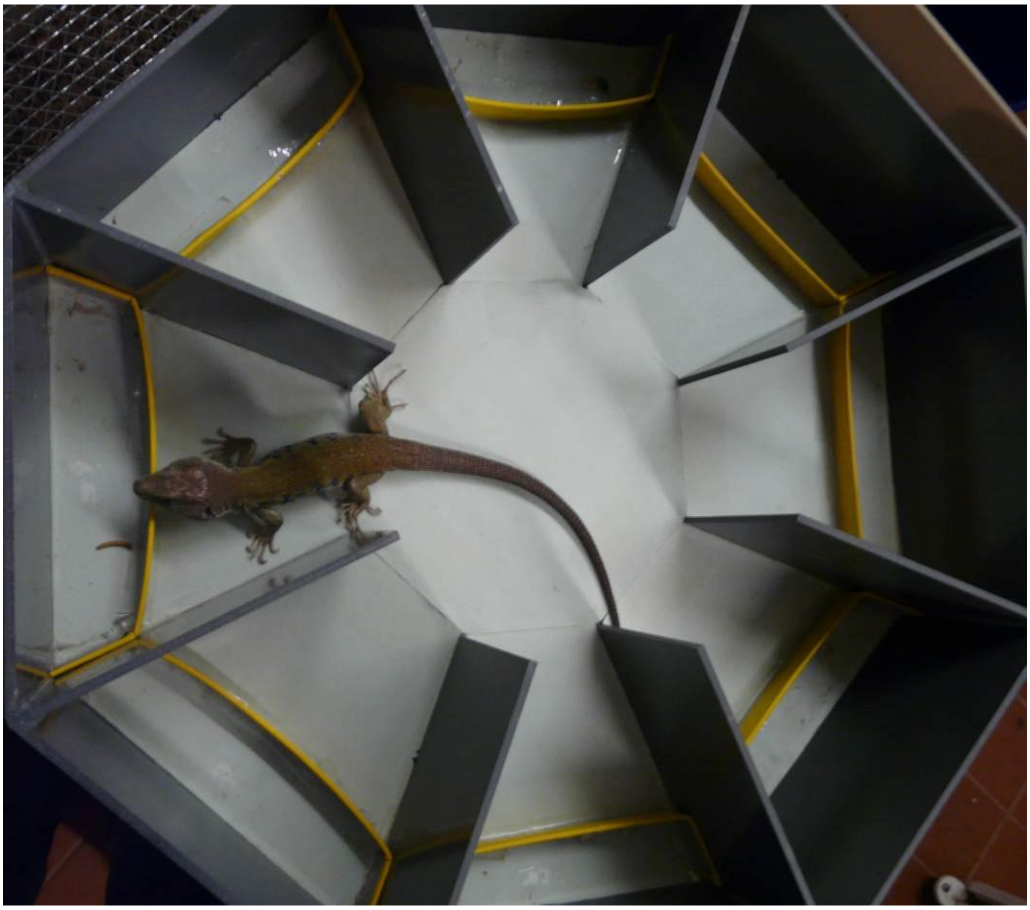

Abstract

Spatial cognition is an essential survival tool that is 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.

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 & 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 et al., 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 & Innis, 1986) or it can be the primary orientation mechanism (Roitblat et al., 1982). Day, Crews, and Wilczynski (1999, 2001) found that three different species of lizard (*Acanthodactylus boskianus*, *Acanthodactylus scutellatus*, & *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 et al, 2011). Members of each class have shown both the ability to navigate based on spatial cues and navigation facilitation relying on response strategies. Wilkinson et al. (2007) showed that a red-footed tortoise (*Geochelone carbonaria*) could successfully master an eight-arm radial maze (Olton & 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 using impoverished distal cues, the tortoise navigated the maze by adopting a strategy of sequentially visiting adjoining arms (Wilkinson et al., 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 & Meyer, 2001).

Methods

Subjects

Three experimentally naïve, captive-bred male jewelled lizards (*Timon lepidus*) of 16 (Barney, sub-adult), 17 (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 terrariums (52x38x40 cm) 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 ± 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 et al. (2009) and Mueller-Paul et al. (in press). 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.

Pre-training: 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. Repeated visits within a trial were considered 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 and discussion

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), $t(15) = 9.235$, $p \leq .001$. However, Barney also performed significantly above chance during the first 20 trials (6.6 novel arms, SD = .88), $t(15) = 7.373$, $p \leq .001$. 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 movement 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, χ^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.

Table 1: Turn type usage of the jewelled lizard compared to chance. Calculation of standardised residuals:

$$\frac{observed\ N - expected\ N}{\sqrt{expected\ N}}$$

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

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 et al., 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 & Samuleson, 1976), appeared to be similar to that of the red-footed tortoise (Wilkinson et al., 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 et al., 1982), and in a red-footed tortoise (Wilkinson et al., 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 & Innis, 1986; humans, Aadland et al., 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 & Innis, 1986). Furthermore, the results match the finding by Day et al. (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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Chapter 5: Social learning in a non-social reptile (Geochelone carbonaria)

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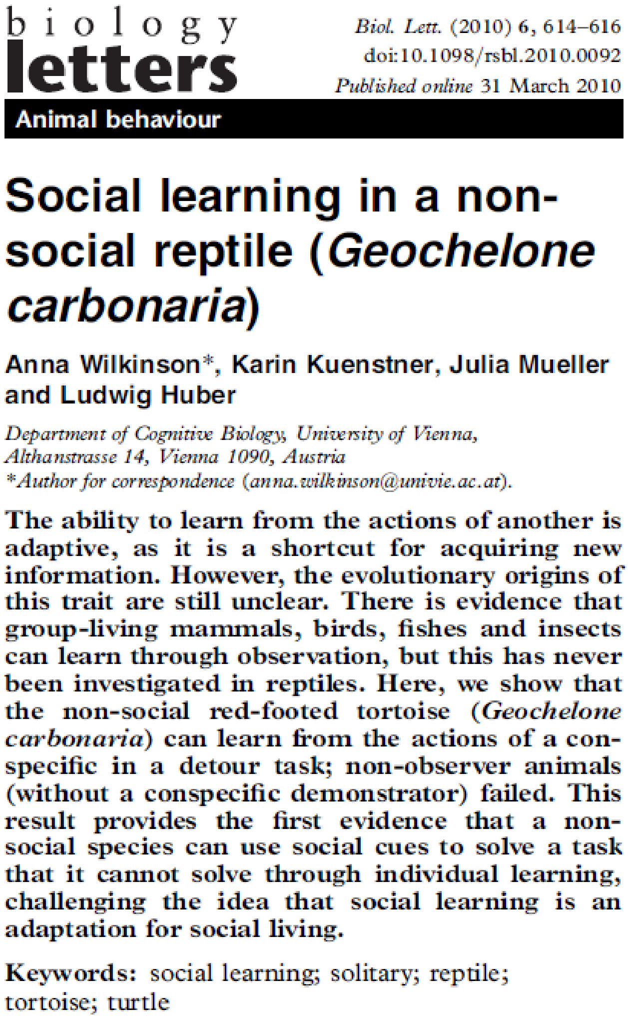

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Animal behaviour

Social learning in a non-social reptile (*Geochelone carbonaria*)

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The ability to learn from the actions of another is adaptive, as it is a shortcut for acquiring new information. However, the evolutionary origins of this trait are still unclear. There is evidence that group-living mammals, birds, fishes and insects can learn through observation, but this has never been investigated in reptiles. Here, we show that the non-social red-footed tortoise (*Geochelone carbonaria*) can learn from the actions of a conspecific in a detour task; non-observer animals (without a conspecific demonstrator) failed. This result provides the first evidence that a non-social species can use social cues to solve a task that it cannot solve through individual learning, challenging the idea that social learning is an adaptation for social living.

Keywords: social learning; solitary; reptile; tortoise; turtle

Abstract

The ability to learn from the actions of another is adaptive as it is a shortcut for acquiring new information. However, the evolutionary origins of this trait are still unclear. There is evidence that group-living mammals, birds, fish and insects can learn through observation, but this has never been investigated in reptiles. Here we show that the non-social red-footed tortoise (*Geochelone carbonaria*) can learn from the actions of a conspecific in a detour task; non-observer animals (without a conspecific demonstrator) failed. This result provides the first evidence that a non-social species can use social cues to solve a task that it cannot solve through individual learning, challenging the idea that social learning is an adaptation for social living.

Introduction

Learning through observing the behaviour of another individual is adaptive as it provides a shortcut to finding a solution, and so avoids the costly process of trial and error learning. There is evidence that group-living mammals (Whiten *et al.* 2005; Huber *et al.* 2009; Heyes & Galef 1996), birds (Zentall 2004), fish (Schuster *et al.* 2006) and insects (Leadbetter & Chittka 2009) can learn socially, however, very little is known about the evolutionary origins of this behaviour despite its prevalence. An almost implicit assumption in the literature is that living in social groups favours the evolution of social learning; this logically leads to the idea that social learning is an adaptation for social living (Klopfer 1961; Templeton *et al*. 1999). The experimental support for this hypothesis, however, is not compelling. The majority of studies investigating the link between social living and social learning have examined social learning abilities in closely related species that differ in sociality. Positive findings (with the exception of Templeton *et al.* 1999) are frequently confounded by species differences in individual learning; those social species that perform better in social tasks also perform better in non-social tasks (see Reader & Lefebvre 2001).

To avoid this problem we approached the question from a different angle, asking whether a non-social animal could learn to solve a task by observing the actions of a conspecific. The adaptive specialisation hypothesis predicts that they could not. However, an alternative hypothesis, which has received little attention in the literature, is that the ability of an animal to learn socially is simply a reflection of an animal's general ability to learn. Any animal with the capacity to learn is likely to be able to use a wide variety of salient stimuli as cues (Heyes 2003); in this context, social cues can be considered similar to other environmental cues and can thus be learned through associative processes. This hypothesis would predict that any species that has had extensive exposure to a conspecific should be able to learn socially.

Using a non-social species provides an ideal scenario for testing the hypothesis that sociality is directly linked to social learning abilities in animals. Truly solitary species within the mammalian and avian classes, however, remain elusive as those that are solitary in their adult life still receive extensive parental care as infants (Galef & Laland 2004). It is therefore unsurprising that only one study has previously used this approach. Fiorito and Scotto (1992) found evidence of social learning in the solitary common octopus (*Octopus vulgaris*). Though apparently compelling, this study has been widely criticised for failing to control for innate species specific behaviour (e.g. Biederman & Davy 1993).

Social learning has never previously been studied in reptiles. Moreover, some species of reptile are solitary and exhibit no parental care (Wilson 1998), making them ideal subjects for this experiment. To this end we examined the social learning abilities of the red-footed tortoise (*Geochelone carbonaria*), a naturally solitary species which inhabits the margins of tropical forests in Central and South America (Strong & Fragoso 2006). Though they may naturally interact with conspecifics (e.g. mating opportunities; Auffenberg 1965), they do not form permanent groups. Indeed, parental care has not been observed in tortoises (Burghardt & Layne 1995): eggs are laid in holes and then left. Once the infants hatch they dig themselves out of the hole and disperse. Despite their solitary nature there is evidence that this species possesses a sensitivity to visual social cues (Auffenberg 1965; Wilkinson *et al.* submitted). This makes the red-footed tortoise an ideal subject for examining whether a solitary species can use the behaviour of a conspecific to reach a goal.

Eight socially housed red-footed tortoises were presented with a detour task. By presenting this task to both non-observer (control) animals, who had to solve the task through individual learning, and to animals who were able to watch a conspecific demonstrator solve the task before they attempted it themselves, we were able to examine the role that social and individual learning plays on solving a task in this species.

Materials and methods

Subjects and Apparatus

Eight red-footed tortoises (*Geochelone carbonaria*) participated in this experiment. Subjects were all juvenile or sub-adult at the time of the study. Individual age was approximated based on inspection of the lower part of their shells (i.e. the plastron), which ranged from 9cm-17cm in length. Though this species of tortoise is naturally solitary, they were group housed for two months prior to the start of this experiment to give them experience with the behaviour of other conspecifics. The tortoises were housed in two groups in a heated (29 ^{o}C ±4 ^{o}C) and humidified room. For the experiment the tortoises were randomly assigned to one of two testing conditions, the non-observer (n=4) and the observer (n=4) conditions. The groups were size (age) matched. Six of the tortoises had previously taken part in experiments (e.g. Wilkinson *et al.* in prep), but were unfamiliar with the present task. Two tortoises were experimentally naïve at the onset of the study. One of the experimentally naïve individuals was in each group.

The task was run in an arena measuring 120cm x 120cm, the floor of which was covered with bark chips. In the centre of the arena was a 40cm high V shaped fence. Each side was 50cm long; the angle between the sides was 110°. The setup was situated in a heated room, maintained at approximately 29^{o}C.

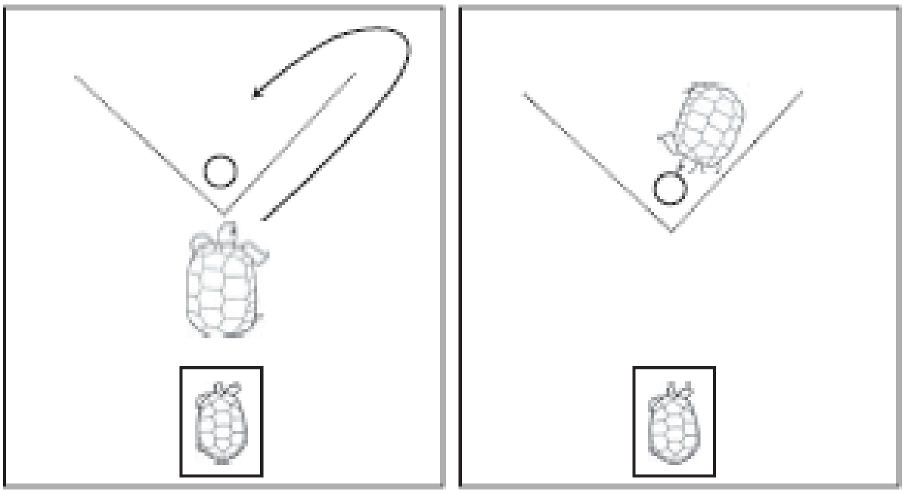

Figure 1: The experimental setup. The arrow indicates the direction in which the demonstrator moved.

Procedure

In each trial a tortoise was placed on the outside of the V-shaped fence and a preferred food was available in the centre. To successfully reach the goal the tortoise had to move away from the food, circumvent the fence and return to the food on the other side (Figure 1 depicts the setup and the experimental group task).

Each animal of the non-observer group received 12 trials (1 per day). For each non-observer trial a tortoise was placed in a small cage in the test arena for 30s. The bark flooring of the setup was redistributed through the entire arena (to avoid any scent trails in the observer condition) before the tortoise was released from the cage. After release each animal was allowed two minutes (from when it started to move) to solve the task. Any animal that reached the goal was allowed to eat the reward.

One of the non-observer animals was then trained, using a successive approximation procedure, to make the detour in a rightward direction. It took over 30 sessions (each consisting of 5 or more trials) to attain reliable performance. The trials of the observer group were identical to those of the non-observer animals except that when each observer animal was placed in the small cage it watched the demonstrator complete the detour and eat the reward. The demonstrator was then removed, the bark redistributed and the reward bowl replenished before the observer tortoise was released. Any trial in which the observer did not watch the demonstration was stopped after the demonstration and repeated. Watching was judged as facing towards the demonstrator for at least 80% of the trial. Any trial in which the demonstrator did not move after 30s was abandoned and repeated later.

Results

None of the four non-observer tortoises reached the goal in any of their 12 trials (Figure 2a). They readily approached the fence in front of the reward but were unable to successfully navigate the detour. However, all four of the observer subjects completed the task at least twice, with two completing the detour on the first trial (Figure 2b). A Fisher exact test comparing the success of each group on an all or nothing measure (whether an animal successfully navigated the detour within 12 trials or not) revealed that the observer group were significantly more successful than the non-observer group, $p<0.05$. The observer tortoises completed the detour in a leftward as well as the demonstrated rightward direction (figure 2b). On their first successful trial three of the observer tortoises went right and one went left. A t-test comparing the percentage of their rightward choices to chance (50%) did not reveal a significant rightward preference $\underline{t}(3) = 0.64$, $p>0.05$.

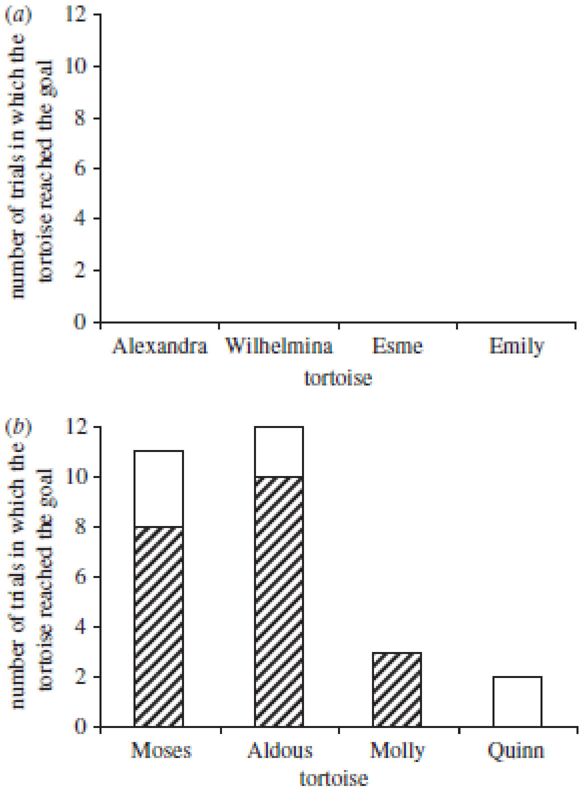

Figure 2: The number of trials in which the tortoises completed the detour and the direction taken for (a) the non-observer group and (b) the observer group. White bars, left; shaded bars, right.

Discussion

The results show that the solitary red-footed tortoise can learn to solve a task by observing the actions of a conspecific. This task was not solved through individual learning. After observing a demonstrator complete the detour all the observer tortoises successfully reached the goal whilst all the non-observer animals failed. Use of a simple strategy, such as following the scent of the demonstrator can be ruled out as the tortoises made the detour in both directions. The results may be explained through local or stimulus enhancement brought about by the presence of a conspecific. This possibility can only be entirely ruled out through use of a two-action procedure (see Zentall 2004). However, we believe that stimulus or local enhancement of some element of the route is unlikely to account for our findings. The demonstration was only made in a rightward direction, but the tortoises' responses were not restricted to this. One of the tortoises went leftward on its first successful detour and two of the other tortoises successfully navigated the detour in both directions. It therefore seems plausible that the tortoises copied some part(s) of the behaviour of the conspecific. This is the first evidence of social learning in a non-social reptile and reveals that sociality is not a prerequisite for social learning.

The dominant hypothesis in this field claims that social learning evolved as a result of social living and therefore predicts that the tortoises would have difficulty with this task. They did not. The findings suggest that, in this case, social learning may be the result of a general ability to learn. Although the brain mechanisms that underlie the tortoises' ability to learn socially remain unclear, it seems most likely that it is the product of a general learning mechanism which allows the tortoises to learn, through associative processes, to use the behaviour of another animal just as they would learn to use any cue in the environment.

To further understand the mechanisms controlling social learning in this species it is necessary to manipulate the amount of social experience that an animal has, as well as to test the importance of the demonstrator being a conspecific (or even an animate object). Further investigation is essential; however, this study provides the first conclusive evidence of social learning in a solitary reptile. The findings suggest that, at least in some cases, the ability to learn socially may simply be a reflection of an animal's general ability to learn.

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Chapter 6: Picture-object recognition in the tortoise *Chelonoidis carbonaria*

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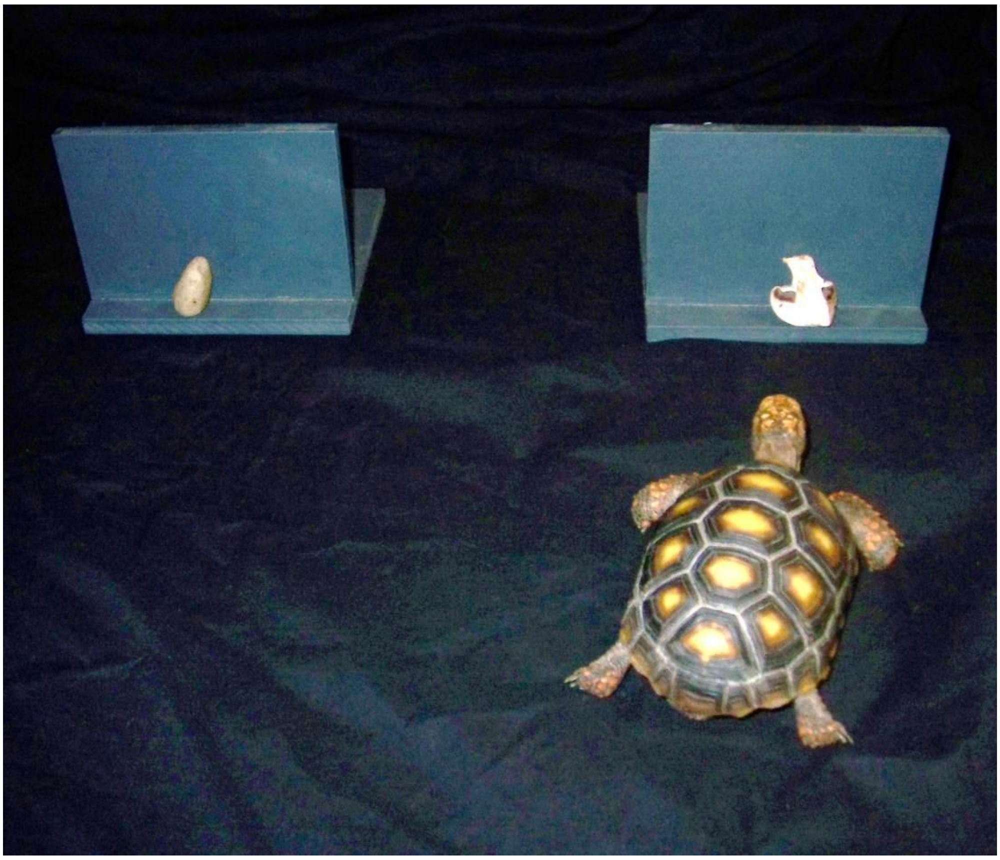

Abstract

To recognize that a picture is a representation of a real life object is a cognitively demanding task. It requires an organism to mentally represent the concrete object (the picture) and abstract its relation to the item that it represents. This form of representational insight has been shown in a small number of mammal and bird species. However, this has not previously been studied in reptiles. This study examined picture-object recognition in the red-footed tortoise (*Chelonoidis carbonaria*). In Experiment 1 five red-footed tortoises were trained to distinguish between food and non-food objects using a two-alternative forced choice procedure. After reaching criterion they were presented with test trials in which the real objects were replaced with color photographs of those objects. There was no difference in performance between training and test trials, suggesting that the tortoises did see some correspondence between the real object and its photographic representation. Experiment 2 examined the nature of this correspondence by presenting the tortoises with a choice between the real food object and a photograph of it. The findings revealed that the tortoises confused the photograph with the real life object. This suggests that they process real items and photographic representations of these items in the same way and, in this context, do not exhibit representational insight.

Introduction

Recognizing that a picture represents an object is an easy, automatic process for humans living in a Westernized society. However, recent research has revealed that this is not necessarily the case for non-human animals or even for all humans (see Bovet and Vauclair 2000 for a review). To truly understand the nature of a picture it is necessary to appreciate that the picture itself is an object that represents another object and to recognize what that other object is (DeLoache 2004). However, understanding that pictures are referential stimuli is not essential for discriminating between two pictorial stimuli. Fagot, Martin-Malivel and Depy (2000) proposed three possible modes of picture perception in animals: independence, confusion and equivalence. In independence processing, an animal fails to relate the picture to real life in any way. It is possible to learn to discriminate pictures by processing them as combinations of features and/or patterns without any recognition of what the picture might represent. The confusion mode of processing refers to the animal perceiving the picture as the real object. It would therefore process the two stimulus types in exactly the same way. The third mode of processing, equivalence, refers to the ability to perceive that the picture is a representation of the object, but is not the object itself.

Evidence of the confusion mode of processing by baboons and gorillas was presented by Parron, Call and Fagot (2008). In a two-alternative forced choice procedure baboons, gorillas and chimpanzees were trained to select a banana in a choice between a slice of banana and a pebble. The primates were then presented with a series of test trials in which one or more of the training stimuli were replaced with photographs. All species chose the slice of banana over the photograph of the banana. They also chose the pictorial banana stimulus over both the real pebble and the photographic representation of the pebble. However, the baboons and gorillas frequently ate the photograph of the banana, suggesting that, despite accurately discriminating between the real fruit and its photographic form, they perceived the banana photograph as a real banana (though maybe a poorer exemplar of one). However, in this study all food related stimuli were yellow and all non-food items were grey. The animals could therefore have solved photographic discrimination by extracting simple color cues rather than visually identifying the object depicted in the photograph. Interestingly, the chimpanzees did not show confusion, suggesting that they at least could exhibit representational insight (equivalence) under these conditions. There is evidence of equivalence in a small number of other species among mammals (Dasser 1987; Kendrick et al. 1996) and birds (Aust and Huber 2006; Aust and Huber 2009; Wilkinson et al. 2010c). However, the nature of this representational insight remains controversial (for reviews see Delius et al. 2000; Fagot et al. 2000; Lea and Dittrich 2000; Watanabe 2000).

The majority of evidence of picture-object correspondence in reptiles comes from work on lizard mating displays (e.g. Macedonia et al. 1994; Ord and Evans 2002; Ord et al. 2002; Van Dyk and Evans 2008) in which male lizards are presented with a video of another male displaying. Many species produce highly stereotyped displays (Jacky dragons: Carpenter et al. 1970; Anolis: Williams and Rand 1977), and video stimuli elicit both threat and submissive displays (Van Dyk and Evans 2008), allow opponent assessment (Ord and Evans 2002) and provide enough information to allow species discrimination (Macedonia et al. 1994). Recent research (Hansknecht and Burghardt, 2010) has revealed that video images of prey can elicit predatory behavior (including attack) in neonate mangrove saltmarsh snakes (*Nerodia clarkii compressicauda*). The reptiles tested in these studies clearly behave toward video stimuli as if they were real conspecifics (or heterospecifics), suggesting that they confuse the video image with a real animal. However, it is not clear what elements of the video stimuli they respond to. It is possible that the lizards' and the snakes' responses were controlled by some element of the movement of the stimuli. If presented with stationary stimuli they may not see any correspondence between the photograph and the object that it depicts. To our knowledge there has been no research investigating static picture perception in reptiles (see Burghardt 1977 for a review of reptile cognitive abilities).

Visual discrimination has been examined in a number of reptile species using a variety of methods. These were extensively covered in Burghardt's (1977) review and there has been little advance in our knowledge since then. More recent work with chelonia (Burghardt and Davis 2007) has revealed that Florida red-bellied cooters (*Pseudemys nelsoni*) can rapidly learn to discriminate between two clear bottles, one of which contained food. Test trials ruled out the possibility that they made the discrimination on the basis of smell. Further, they were able to retain this discrimination over two years of non-exposure (Davis 2009). A follow up experiment (Davis 2009) revealed rapid learning of a black vs. white discrimination. This learning was retained for 2 months after training had ceased (it is likely that they are able to retain this for longer, though this was not tested).

The aim of this study was to investigate how a reptile, the red-footed tortoise (*Geochelone carbonaria*) perceives static pictures. The red-footed tortoise is an omnivorous species native to Central and South America. In comparison to other tortoise species it is relatively active (Moskovits 1985, cited by Strong and Fragoso 2006) and thus ideal for this kind of experiment. Though research on color perception has not been conducted with this species, work on another chelonian (*Pseudemys scripta elegans*) and also on goldfish and birds has revealed that they possess tetrachromatic vision (Neumeyer 1998), which makes it likely that the same is true of the red-footed tortoise. To our knowledge no psychophysical data exist on the visual perception of this species. However, the small amount of cognitive research that has been conducted with the red-footed tortoise suggests that vision is extremely important to this species. For example, in a radial arm maze task the red-footed tortoise navigated using visual cues over olfactory ones (Wilkinson et al. 2007). When visual cues were unavailable the tortoise used a highly successful response based strategy. However, as soon as access to visual cues returned the tortoise changed its behavior and started to use visual cues for navigation (Wilkinson et al. 2009). To date only one individual of this species has been reported to have chosen smell as a cue in only one single experiment when visual cues were made unavailable (Mueller-Paul, Wilkinson, Hall & Huber in press). Further, social tasks have revealed that this species can follow the gaze direction of a conspecific (Wilkinson et al. 2010b) and is able to learn to access an otherwise inaccessible goal by observing the actions of a conspecific (Wilkinson et al. 2010a). Thus their relatively high activity levels, highly developed visual system, and reliance on visual cues makes them an ideal species for investigating picture-object recognition in reptiles. We therefore presented five red-footed tortoises with a picture-object recognition task reminiscent of that used by Parron et al. (2008).

Experiment 1

Experiment 1 examined whether or not the red-footed tortoise could learn to discriminate between food and non-food objects in a two-choice task. The tortoises were trained to approach the food and were rewarded for doing so by being allowed to eat it. To discourage the tortoises from using a simple perceptual rule in the test trials (e.g. choose yellow, as could have been used by the primates in Parron et al. 2008) twelve stimuli that differed in terms of shape color and texture were used. They were matched for color and size so that no one single cue could control behavior.

To test for the different modes of picture-object processing the tortoises were presented with photographs of the items that they had learned to discriminate. If the tortoises could not spontaneously perceive any relation between the photograph and the object that it represented then it was expected that performance would fall to chance levels. However, if the tortoise either confused the photograph with the real item or perceived the photograph as a representation of the real item then it was expected that performance would remain high.

Methods

Subjects

Five captive-bred red-footed tortoises (*Chelonoidis carbonaria* formerly *Geochelone carbonaria*) participated in this study. The exact age of the tortoises was unknown; however, all were juvenile or sub-adult with plastron (the lower part of the shell) lengths measuring between 9cm and 17cm and a minimum age of 3 years at the start of the experiment. The sex of some of our subjects was unknown as this species does not develop unambiguous sexually dimorphic traits until around the age of 5 (see Table 1 for individual sizes and sex, where known). When not taking part in the experiment the tortoises were housed in two groups in a heated (29 ^{o}C ±4 ^{o}C) and humidified room. They were kept on a daily 12L:12D cycle (light on 08:00–20:00) and had access to food (fruit and vegetables) in the evening after experimentation was finished. None of the tortoises were experimentally naïve (e.g. Wilkinson et al. 2007; Wilkinson et al. 2009; Wilkinson et al. 2010a; 2010b), but they had never previously been involved in a discrimination task and had never been presented with photographic stimuli before.

Table 1: The size, sex (where known) and positive stimuli of each tortoise.

Tortoise	**Size (cm)**	**Sex**	**+ Stimuli**	**- Stimuli**
Esme	11	Unknown	Cucumber Melon Rocket Tomato	Pebble Red & green scourer Yellow & green scourer Bottle top Tree branch Red sponge Green sponge Green paper
Molly	11	Unknown	Cucumber Kiwi Melon Tomato	Green sponge Pebble Red sponge Green paper Yellow & green scourer Tree branch Red & green scourer Red paper
Aldous	17	Male	Kiwi melon, plum rocket	Bottle top Yellow & green scourer Red & green scourer Red paper Tree branch Red sponge Green sponge Green paper
Wilhelmina	16.2	Female	Cucumber kiwi melon tomato	Green paper Red sponge Green sponge Pebble Bottle top Yellow & green scourer Red & green scourer Red paper
Moses	14	Female	Kiwi Melon Rocket tomato	Tree branch Red sponge Green sponge Pebble Bottle top Yellow & green scourer Red & green scourer Red paper

Apparatus

The study was conducted in an arena measuring 80 x 100 cm in a heated room, maintained at approximately 29^{o}C. It was lit by two 38 W fluorescent tube lights positioned behind a grid in the ceiling to the right of the experimental apparatus. At one end of the tank were two presentation tables positioned 20 cm apart. Each table consisted of a base platform that was 15 cm x 15 cm and made of grey plastic. Attached to this at a 90^{o} angle (making an inverted T shape) was another piece of grey plastic that measured 10 cm x 15 cm. The tables were used to present the stimuli throughout the experiment. At the opposite end of the tank was a small cage measuring 18 x 27 x 21.5 cm. At the onset of a trial the tortoises were placed inside the cage. The cage was 50cm away from both presentation tables (see Figure 1 for a diagram of the experimental setup). The floor of the tank was covered in bark to ensure that the tortoises could readily move around the apparatus.

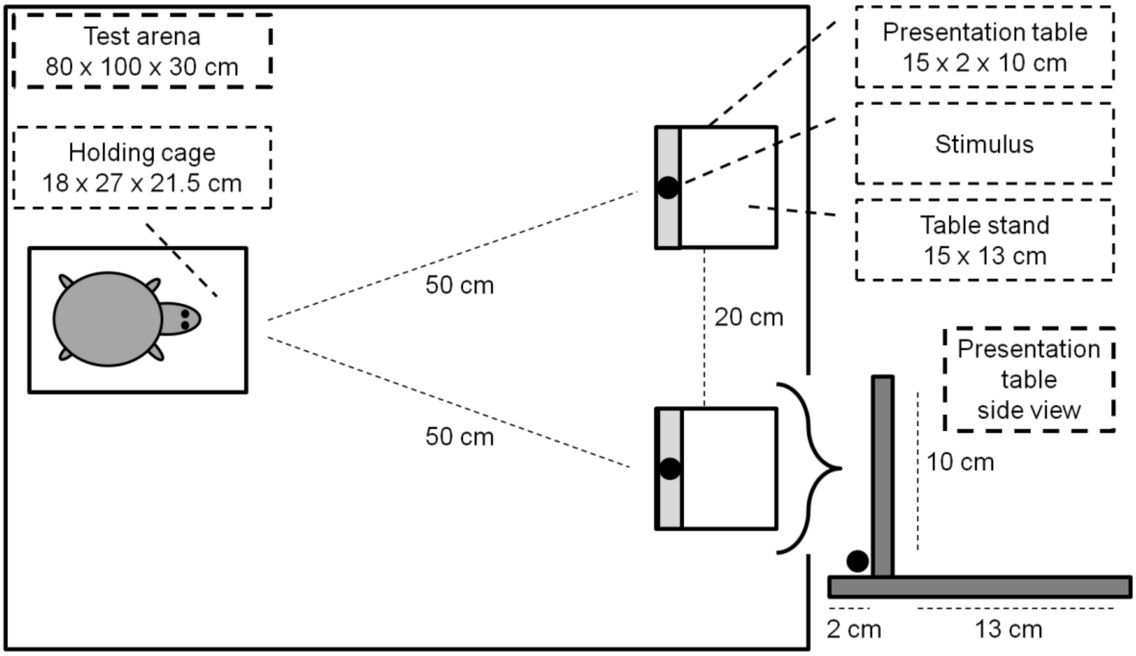

Figure 1: Diagram of the experimental setup showing the testing arena with a tortoise before release from the holding cage on the left hand side and the presentation tables with stimuli on the right hand side.

Stimuli

The stimuli consisted of 6 different food objects (fruit, vegetables and salad) and 12 different non-food objects. However, each animal was only trained on 4 food objects and 8 non-food objects. The choice of the particular stimuli presented to each animal was made on the basis of individual food preferences; see table 1 for details of the specific stimuli received by each tortoise. The non-food objects were selected to ensure that they were similar to the food objects in color, shape and/or texture so that the photographic test stimuli could not be discriminated on the basis of a single cue. All stimuli were presented on a grey background. Test stimuli consisted of two different photographs (JPEG, 2514 x 1721 pixels, 72dpi, 24 bit depth, sRGB, printed at 10 x 15 cm, taken by a Panasonic DMC-FZ18) of each training stimulus. During the test sessions the intermixed training stimuli were presented with a grey photograph behind them to ensure that the tortoises did not use the slight difference in background as a cue to stop performing because the test trials were not reinforced.

Procedure

General Procedure: The experiment took place over a six month period and trials were run five days per week (M-F) and took place between 10am and 4pm. The tortoises were tested individually in the experimental tank. A two-alternative forced choice procedure was used. A trial started with the tortoise being placed into the small wire cage. The tortoise was released after it had looked at (oriented its head towards) both presentation tables. Upon release the tortoise had one minute to approach one of the presentation tables. A choice was counted if the tortoise approached within 5cm of the table. Only one choice was allowed. Trials were separated by an inter-trial interval of at least 5 minutes.

Training: On each training trial the tortoise was presented with a food object and a non-food object, one on each presentation table. Both the side of presentation and the combination of food and non-food objects was counterbalanced across trials to ensure that every food object was paired with every non-food object an equal number of times. Scent cues were controlled for by rubbing the table on which the non-food object was presented with chopped food. This ensured that smell of the reward was present on both presentation tables. If the tortoise chose the food item, the trial was counted as correct, and the tortoise was allowed to eat the piece of food. If the tortoise chose the non-food object the trial was counted as incorrect and the tortoise was removed from the apparatus. The tortoises received four training trials per session. Training continued for at least 12 sessions and until the tortoises chose the food item on at least 13 out of the last 16 trials.

Testing: Test trials were identical to training trials except that the real stimuli were replaced with photographs of the stimuli. As only photographic stimuli were presented, chopped food was rubbed on both tables. Two test trials were presented per session; they were intermixed with four training trials resulting in a total of six trials per test session. The tortoises received a total of 16 test trials. This resulted in a photograph of each of the four food items being presented four times and a photograph of each of the eight non-food object being presented twice. In every test trial the photograph of the food item was presented in combination with a different photograph of a non-food object. Test trials were not reinforced.

Results and discussion

Training: Table 2 presents the individual learning data for each tortoise. The percentage of correct choices in a session was calculated for each individual. A within subjects t-test comparing the percentage of correct choices in the first two sessions to the last two revealed a significant difference, $\underline{t}\,(4) = -5.72$, $\underline{p} \leq 0.01$, this suggests that the tortoises learned to discriminate the food from the non-food object. It is also possible that this difference was the result of habituation to the setup; however, this does not impact upon the interpretation of the test data. The tortoises readily approach the real food item and performed to criterion after an average of 12.8 sessions (± 0.58 SE; Table 2). A one sample t-test on all the training data revealed that overall choice behavior differed significantly from chance, t (4) = 9.88, p ≤ 0.001.

Table 2: The number of correct trials (out of a possible 4) for each tortoise on every training session.

Session	Esme	Molly	Aldous	Wilhelmina	Moses
1	3	3	3	1	3
2	3	3	2	3	4
3	3	4	3	2	4
4	4	3	3	3	2
5	2	1	2	3	4
6	3	3	4	2	2
7	4	3	4	3	4
8	4	4	3	3	3
9	3	3	4	2	3
10	3	3	3	3	3
11	4	3	3	4	4
12	3	3	4	2	4
13		3		4	
14		3			
15		4			

Testing: Figure 2 shows the tortoises' performance on the test trials and intermixed training. The tortoises readily discriminated between the photographs of the food and non-food objects. A one sample t-test revealed that their performance on the test trials was significantly above chance, t (4) = 5.66, p ≤ 0.01. Further, a paired samples t-test revealed no difference in success levels between the training and test trials t(4) = 2.10, p > 0.05.

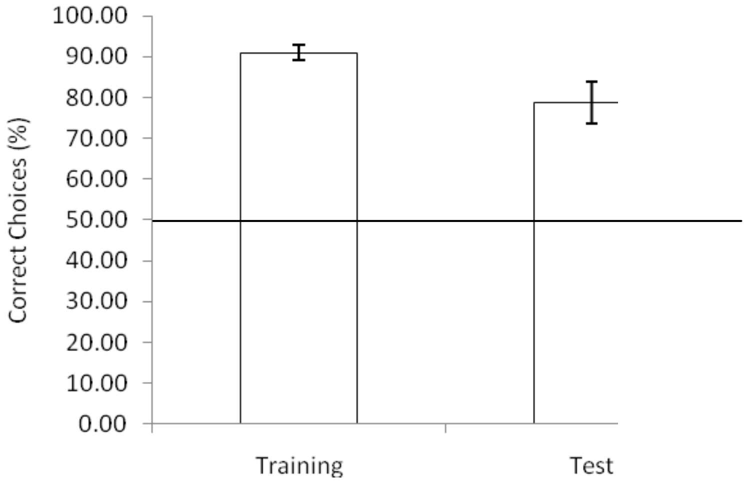

Figure 2: Experiment 1. The percentage of correct choices on the test trials and the intermixed training trials. In these test trials the real objects were replaced with photographs. The tortoises were not rewarded for the choice on these test trials. The whiskers represent the standard error.

The results of this experiment suggest that the tortoises were able to see a correspondence between the photographic stimuli and the real-life objects. Their performance differed little between the training and test trials. However, from this experiment it is not clear how they actually perceived the photographic stimuli. It is possible that the tortoises recognized that the picture, though an object itself, represented another object, thus possessing representational insight. However, it is equally likely that the tortoises could not tell the difference between the photographic stimuli and the real stimuli.

Experiment 2

Experiment 1 revealed that the tortoises were able to see that the photographic images in some way corresponded to real objects. This rules out the possibility that they saw the photographs as combinations of features or patterns that were entirely independent of the object that was represented. However, it is unclear whether they viewed the pictures as a representation of the real object or perceived them to be the same thing. The photograph and the real food item share many cues such as color (though this may differ given the tortoises visual system), size, brightness, texture. However, they also lack many cues that are present in the real food item, such as 3D information and smell. To examine what the tortoises perceived in the photographic images they were presented with a set of tests in which they had a choice between the real food item and a photograph of it. If the tortoises always select the real food item then it suggests that they can see a correspondence between the picture and the real item, whilst still being able to see the differences between them. However, if the tortoises do not reliably choose the real food item then it suggests that they confuse the picture with the real food. These tests were presented in two phases, in Phase 1 there was no differential reinforcement and the tortoises were not rewarded for either choice. However, in Phase 2 the tortoises were rewarded for choosing the real food item.

Methods

Subjects

The tortoises from Experiment 1 participated in Experiment 2.

Apparatus

The apparatus was the same as in Experiment 1.

Procedure

Testing, Phase 1. Phase 1 of testing began immediately after the completion of Experiment 1. All tortoises maintained good performance throughout the first test and therefore required no retraining before the start of Experiment 2. As with Experiment 1 two test trials were presented per session; they were intermixed with four training trials which resulted in a total of six trials per test session. Tests were implemented in exactly the same manner as Experiment 1. However, the tortoises were presented with a real food item on one of the tables and a photograph of that food type (though not that exact piece) on the other table. Their task was to approach one of the stimuli. There was no differential reinforcement. If the tortoise chose the real food item it was removed before it could eat the food. In all other respects the testing procedure was identical to that of Experiment 1.

Testing, Phase 2. Phase 2 of testing began immediately after completion of Phase 1. Testing was identical to that of Phase 1 with the exception that if the tortoises chose the real food item they were allowed to eat it. If they chose the photograph of the food item they were removed from the apparatus. Thus, they were differentially reinforced for choosing the real food item.

Results and discussion

Phase 1: The percentage of correct choices in a session was calculated for each individual (figure 3). A t-test comparing the tortoises' performance in the test trials to chance revealed that there was no difference, $\underline{t}\,(4) < 1$. Further, performance on test trials was significantly poorer than on the intermixed training trials, $\underline{t}\,(4) = 5.04$, $\underline{p} \leq 0.01$. This is surprising given that a real food item has a number of highly salient cues that photographs do not, e.g. smell, (though we did try to control for this) and 3D information. It is therefore possible that, rather than being unable to differentiate between the photograph of the food and the real food, the tortoises had learned that there was no differential reinforcement on the test trials and thus performed at chance levels. Phase 2 tested whether differentially reinforcing the test trials would improve the tortoises' performance. If the tortoises were truly unable to discriminate the real food from the photograph then differential reinforcement should have no impact upon performance.

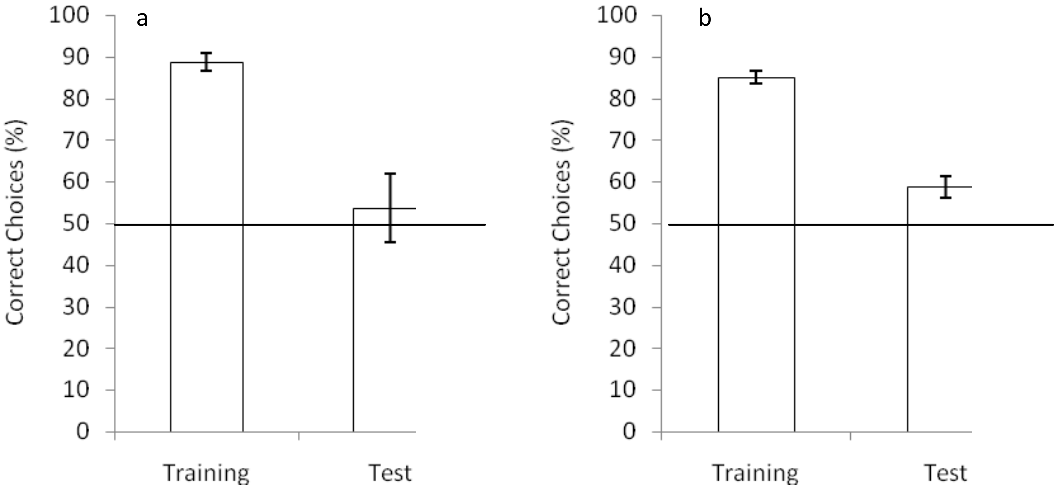

Figure 3: Experiment 2. The percentage of correct choices on (a) Phase 1 test trials and intermixed training trials. In these test trials the tortoises were presented with a real food item vs. a photograph of that food type and were not differentially reinforced for the choice. (b) Phase 2 test trials and intermixed training trials. In these test trials the tortoises were presented with a real food item vs. a photograph of that food item and were rewarded for choosing the real food item. The whiskers represent the standard error

Phase 2: Examination of Figure 3b reveals that differential reinforcement did slightly improve the tortoise's performance on the test trials. A t-test comparing their scores to chance levels revealed a significant difference, $\underline{t}$ (4) =3.5, $\underline{p} \leq 0.05$. However, the mean percentage correct was only 59% on the test trials and performance on the test trials remained significantly below that of intermixed training trials (that presented a food and a non-food object), $\underline{t}$ (4) = 9.64, $\underline{p} \leq 0.001$. Further, there was no significant difference between the tortoises' performance during the Phase 1 test trials and those of Phase 2, $\underline{t}$ (4) < 1. This suggests that, although the tortoises were able to discriminate between the photograph and the real item when reinforced differentially, it was difficult for them to do so.

General discussion

The experiments presented here demonstrate for the first time that reptiles are able to perceive the imagery on a photograph and the object that it represents in a similar manner. The tortoises were trained to discriminate between a series of food and non-food objects. They rapidly mastered this task and retained high levels of discriminative performance when the real items were replaced by photographs. This suggests that they recognized some elements of the real objects in the photographs. Experiment 2 tested whether the tortoises processed the photographs in the mode of confusion or equivalence. The tortoises were presented with a real food item versus a photograph of the same food. They did not discriminate between the two in initial testing; however, when differentially reinforced they chose the real food item significantly more than would be expected by chance.

Experiment 1 provided compelling evidence that tortoises are able to transfer a learned discrimination from real stimuli to the photographic equivalent. The tortoises behaved towards the photograph and the real object in a similar way, suggesting that the tortoises did not process photographs as a collection of irrelevant patterns. However, the data from Experiment 2 suggests that the tortoises did not see the photographs as representations of the stimuli but rather mistook them for the real objects. This level of processing is in accordance with Fagot et al.'s (2000) second mode, confusion. This is surprising given that pictures lack multiple cues that real items possess. Further, the photographs used as stimuli were created for human trichromatic vision. It is probable that the red-footed tortoise has tetrachromatic vision and as a result photographs are likely to be even more impoverished representations of the real item for tortoises than they are for humans.

It is surprising, given the general importance of chemical cues among many reptiles, that odor cues from the food did not influence the tortoises' choice behavior in Experiment 2. This contradicts some recent work in which the presentation of both visual and odor cues together resulted in a stronger response in salt marsh snakes than would have been predicted as the result of the summation of the two stimuli when presented alone (Hansknecht and Burghardt 2010). It is possible that during training the tortoises had learned to ignore odor cues and thus did not use them in the later tests. However, it provides further interesting evidence of the differences between different species (and potentially orders) of reptiles and lend support to the idea that vision may be a dominant sense in the red-footed tortoise. This finding is not related to a specific test but appears over a variety of experimental tasks. It has been observed in both the spatial (Wilkinson et al, 2007; 2009) and the social (Wilkinson et al. 2010a; 2010b) domain in number of different setups. The tortoises did not show any indication that they relied on their sense of smell at all during this experiment. This idea is also supported by the findings of Burghardt and Davis (2007) and Davis (2009) who found no effect of smell on a visual discrimination task with Florida red-bellied cooters and suggests that this may be common to all chelonia.

Although the tortoises did exhibit an improved performance in Experiment 2, Phase 2 it seems unlikely that a lack of reward in the test trials was controlling their behavior in Phase 1. If this had been the only reason for poor performance then it would be expected that the tortoises would perform equally as well as they did in the test session of Experiment 1. Though performance did increased slightly during Phase 2 it remained significantly below performance on the intermixed training trials and did not differ significantly between the tortoises’ correct choices on the test trials in Phase 1. This suggests that the tortoises had great difficulty in distinguishing between the photograph and the corresponding real item and that their improved performance was the result of further experience. Though the tortoises were able to recognize a difference between real items and photographs of them to some extent, the level of cognitive processing of these images by the red-footed tortoises is indicative of the confusion mode of processing (Fagot et al. 2000). Interestingly, very similar behavior was observed in the baboons and gorillas by Parron et al. (2008). They suggested that the baboons and gorillas processed the picture of a banana as a poor, less attractive, but nevertheless real exemplar of the banana category. It seems that for the tortoises the picture represented an exemplar of the “food” category, but, while it was not identical, it was not necessarily much poorer than the real food item.

The methodology used in this experiment was based on that used by Parron et al. (2008). It could be argued that using these methods enhances the likelihood that the animals will confuse the picture and the object. For example, the photographic stimuli were the same size as the real objects that they represented. This is unusual in these types of experiments and most previous experiments present the pictured item much smaller than it would be in real life (e.g. Aust and Huber 2006; Wilkinson et al. 2010c). However, this method ensures that the animals do not learn the task on the basis of an extraneous cue such as size. The second similarity between the present study and that of Parron et al. (2008) was that all animals used in the two studies were naïve to pictures. They had never been trained on, or presented with, photographic stimuli. It is possible that, given extensive exposure to photographs the baboons, gorillas and tortoises may be able to learn that the photographs are not the real object, but merely representations of it.

This study has shown that tortoises can discriminate between pictures of real objects according to previously learned parameters. However, the specific elements of the photograph that were necessary for this remain unclear. Categorization can be based on an enormous array of visual features (for recent reviews see Huber 2010; Huber and Aust in press). A next step would be to manipulate the stimulus parameters of the photographs to investigate what controlled the tortoises’ discriminative behavior. This could be done in terms of color, shape or specific features and would allow comparison with other species whose picture perception and visual categorization abilities are better understood.

There has been much recent debate on the importance of picture perception in the interpretation of visual discrimination tasks across a number of species (see Comparative Cognition and Behavior Reviews, 5, 2010). The findings of the present experiment suggest that when a red-footed tortoise is presented with pictorial stimuli in a visual discrimination task it will perceive the stimuli as exemplars of their referent. This has important implications in terms of the interpretation of the data produced in visual discrimination and categorization and suggests that the red-footed tortoise may be an ideal species for future work with reptiles in this area.

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Chapter 7: Red-footed tortoises (*Geochelone carbonaria*) are capable of transferral between the touch screen and a physical arena

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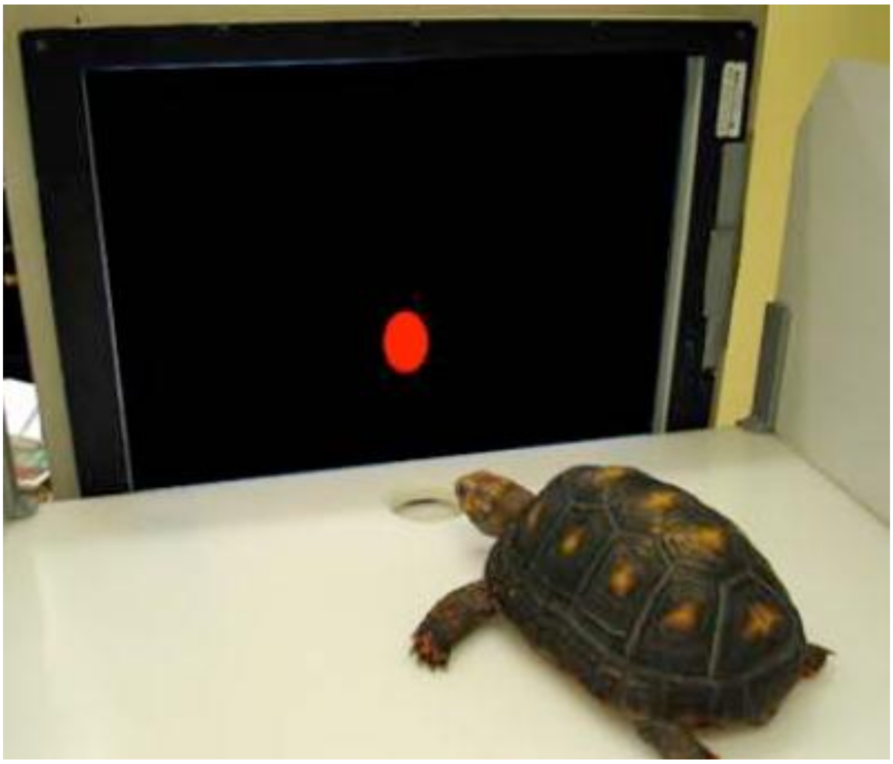

Abstract

In recent years red-footed tortoises have been shown to be proficient in a number of spatial cognition tasks, where cognitive map-like behaviour, response stereotypy were observed further, they perceive a correspondence between real objects and 2D images of them. The present study made use of their 2D-image recognition ability in order to further investigate the patterns underlying tortoise spatial navigation. We tested whether tortoises were able to transfer spatial knowledge acquired in one domain to another domain. Four red-footed tortoises were subjected to a simple spatial task presented on a touchscreen. Upon learning this they were tested in a physical arena, followed by reversal learning in an arena and a test on the touchscreen. The results show, for the first time, that red-footed tortoises are able to operate a touchscreen and can successfully solve a spatial two choice task in this apparatus. Furthermore the results show that information learnt on the touchscreen is reliably transferred to a physical arena that provided a comparable setup. Thus, the use of touchscreens is a viable method in testing these tortoises and has the potential to greatly widen the possibilities for studying this species.

Introduction

The ability to navigate through space successfully and efficiently can be considered a significant survival advantage as it allows for the safe passage between feeding grounds, sleeping quarters and hiding places. Most research on spatial cognition has concentrated on the navigation of mammals and birds (Thinus-Blanc, 1996). Far less research has investigated this in reptiles and within the area of reptile spatial cognition there is a clear emphasis on the study of seasonal, large scale movements of sea turtles (Dutton, Bowen, Owens, Barragan, & Davis, 1999). However, most reptiles spend their lives in much smaller scale environments which are familiar to the animals. Different strategies of short-scale navigation in reptiles have been identified experimentally, such as cognitive-map-like navigation, orientation based on a single salient landmark (beacon), and response stereotypy (for a review see Mueller, Wilkinson, & Hall, 2011).

Holtzman, Harris, Aranguren, and Bostocks (1999), for example, showed that corn snakes (*Elaphe guttata guttata*) were able to learn the position of a goal amongst false goals when provided with one single, obvious landmark called a beacon. The terrapin *Pseudemys scripta* can use extra-maze room cues to find a specific location in a T-maze, regardless of the starting position. Interestingly, their performance was not diminished by obstructing half of the available room cues but was disabled when all room cues were hidden. This suggests the use of a cognitive-map-like strategy (López et al., 2000). In a further study López et al. (2001) found that the same terrapin species was capable of some flexibility in their use of navigational mechanism. They trained one group which only had access to extra-maze cues to learn to navigate to the goal. A second group was provided with the same extra-maze cues but also with a prominent intra maze beacon. While both groups successfully learnt to navigate to the goal the beacon was used when present.

Red-footed tortoises and jewelled lizards have been shown to master an eight-arm radial maze, which required them to remember several different spatial locations within a single trial (Mueller-Paul, Wilkinson, Hall, & Huber, in press; Wilkinson, Chan, & Hall, 2007). Red-footed tortoises appear to be able to use room cues for navigation in a cognitive map-like manner but also exhibit stereotypic response strategies if cues are less salient (Mueller-Paul, Wilkinson, Hall, & Huber, in press; Wilkinson, Coward, & Hall, 2009). Odour, too, has been identified as a possible cue, but appears to be only used when other cues cannot be used. However, even though the tortoises are clearly able to change strategies when required by the task, they appear to prefer the first successful strategy they have discovered, even if another might be easier under changed circumstances (Mueller-Paul, Wilkinson, Hall, & Huber, in press).

In another set of studies, Wilkinson, Kuenstner, Mueller, and Huber (2010a) found that red-footed tortoises can learn a spatial path that leads to a goal by observing a demonstrator tortoise. It appears that in this task the tortoises did not only learn about the exact route shown by the demonstrator but were able to apply the principles of the task even when the path to food was altered (Wilkinson & Huber, in press). Thus, red-footed tortoises have demonstrated the ability to generalise knowledge across variations of a task they have learnt.

To further test their ability to generalise it is necessary to test the tortoise's performance on comparable tasks in very different domains, such as the touchscreen versus a traditional testing arena. Differences and similarities of behaviour in such distinct domains are important for determining the generality of spatial cognitive processes in red-footed tortoises. Spontaneous transfer of knowledge in one domain to another would indicate a high level of generality of the spatial skills learnt. Transfer from the touchscreen to a 3D arena would indicate that a kind of mental map provided by the overview of the touchscreen encourages accumulation of broad, generalisable knowledge. Transfer from the arena to the touchscreen in contrast would suggest the same for a more active, sensori-motor based approach to learning.

Very little research has directly compared performance in touchscreen and 3D tasks. O'Hara (in prep) found that kea (*Nestor notabilis*) could learn a comparable task both on the touchscreen and in a 3D, 'reality' version of it. However, learning was significantly faster in the reality condition than on the touchscreen. The authors conclude that different mechanisms might be underlying the two learning paradigms while the difference might also reflect the kea's great liking of objects for play, which might have lead to higher motivation levels during the reality task. O’Hara’s results emphasise the importance of testing animals in different domains to form a complete picture of the species’ abilities. Strong similarities between spatial learning performance on a touchscreen and in a 3D arena have been demonstrated in pigeons (*Columba livia*, Kelly & Spetch, 2004; Kelly, Spetch, & Heth, 1998). Kelly and colleagues demonstrated that pigeons were able to use featural and geometric cues to a similar extend when presented in a 2D schematic and in a navigable 3D environment. This implies that similar spatial learning mechanisms govern the performance in these different domains, at least in pigeons.

Transferral between the touchscreen and a 3D arena is particularly interesting as it allows for the analysis of the mechanisms used in two distinct domains which differ in a number of important points, while asking essentially the same questions. Touchscreen experiments usually present their stimuli on a much smaller scale allowing the animal to oversee the entire task which they are required to solve while this is not necessarily the case when the animal needs to move around a 3D experimental setup. This overview of the task might facilitate learning. However, the fact that birds (Kelly et al, 1998; Kelly & Spetch, 2004) which, through their ability to fly, are familiar with different perspectives and the overview, “birds-eye” perspective in particular, are capable of mastering 2D presentations of tasks does not necessarily predict that this ability extends beyond this class. The physical presence within the task environment is more naturalistic and presents information based on changes in perspective and sensori-motor feedback which might be advantageous or even necessary for learning.

One prerequisite for the use of touchscreens, the ability to perceive and understand 2D images, has been demonstrated in a recent study by Wilkinson, Mueller-Paul, and Huber (submitted) showing that red-footed tortoises were able to recognise a correspondence between real objects and 2D images of them. Tortoises that were trained to distinguish colour-matched food and non-food items were later able make the same distinction between colour photographs of similar food and non-food items. Furthermore, the tortoises had difficulties distinguishing between the real food items and the photographs of food items, suggesting that they might process 2D and 3D stimuli in a similar manner. A further prerequisite for touchscreen work, the ability to peck at an arbitrary response location when triggered by a stimulus in order to receive a reward in a different feeder location, has been demonstrated in terrapins (*Chrysemys picta picta*) by Bitterman (1964). The terrapins were proficient enough in the use of pecking keys to obtain their entire daily food allowance by means of the pecking apparatus for an extended period of time.

The present study made use of the 2D-image recognition ability of red-footed tortoises in order to further investigate the mechanisms underlying tortoise spatial navigation. To this end, performance in a touchscreen task that provided small scale stimuli and a full overview of the situation was compared to that in a 3D arena that required walking through space towards a physical stimulus thus providing stronger motor feedback. We tested for transfer of knowledge acquired in one domain to the other. Four red-footed tortoises were subjected to a spatial task presented on a touchscreen and tested in a physical arena, followed by reversal learning in an arena and a test on the touchscreen.

Methods

Subjects

Four juvenile red-footed tortoises (*Chelonoidis carbonaria* – formerly *Geochelone)* with plastron lengths of 13 cm (Esme), 13 cm (Molly), 12 cm (Quinn) and 11 cm (Emily), took part in the study. The tortoises' sex was unknown, as unambiguous sexual dimorphism develops only develops later in the life of this species. The tortoises were housed as a group of four in a 120 x 70 cm arena, at 28 +/- 2°C and approximately 60% humidity, with permanent access to fresh water, shelter, UV light, and heat lamps. The tortoises were not food deprive, small pieces (approximately 0.5 x 0.5 cm) of preferred fruit and vegetables, such as mushroom, strawberry, and sweet corn were provided as rewards during experimental session while a variety of less preferred food types, such as cucumber, grape, and apple was offered in their arena after training. In accordance with standard husbandry practice they experienced one day a week without food. All four animals had previous experimental experience (see Mueller et al, 2011; Wilkinson et al., 2010a; 2010b; Wilkinson, Mueller, & Huber, 2011) but they had never previously been trained on a touchscreen, pecking key or similar apparatus.

Apparatus

Touchscreen apparatus: A 38cm Videoseven touchscreen with a resolution of 1024 x 768 pixel and 32 bit colour quality was used. The computer was self-made with 999MHz and 480 MB RAM, running Microsoft Windows XP professional. The software controlling the touchscreen apparatus was CogLabLight1.9-B57.

The touchscreen was placed in a 30 x 50 cm Skinner box (*figure 1*) with 21 cm high white plastic walls and a floor covered with grey grip-ensuring rubber lining. It was open at the top. Centrally, at a distance of 2 cm from the touchscreen, a feeder hole was positioned in the floor of the box. Underneath the floor the feeder apparatus was attached to the touchscreen box. The feeder mechanism was a round plate with a diameter of 47 cm and 16 small indentations indicating the reward positions around the outer edge. A correct response resulted in the feeder plate turning by one reward position which resulted in a reward being presented below the feeder hole, making it accessible for the tortoise.

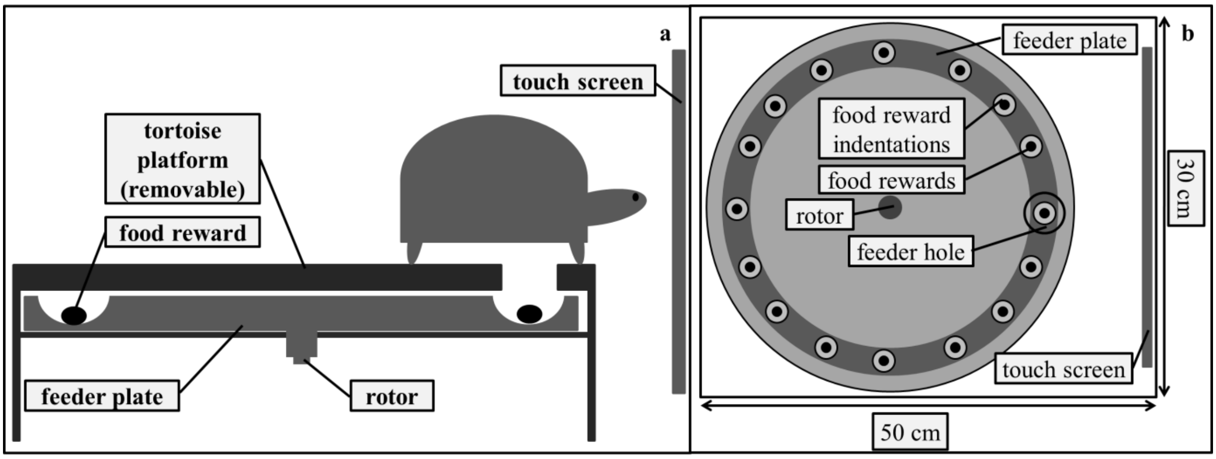

Figure 1: Tortoise touchscreen box: (a) side view of the touchscreen box, (b) top view of the feeder plate (illustrations not drawn to scale)

The touchscreen apparatus stood in the centre of a 2.24 x 2.24 m room in the Department of Cognitive Biology at the University of Vienna, Austria. The room was lit with two 25-W fluorescent tube lights and the walls contained a variety of posters.

Arena apparatus: The arena was 100 x 80 x 40 cm with the bottom 20 cm of the side walls covered with white paper and the upper 20 cm being of see-through glass. The floor was covered in beige wood shavings. This apparatus was positioned in a different room (2.28 x 2.24 m) and in a different spatial orientation from the touchscreen apparatus. The room was lit by two 25-W fluorescent tube lights. Furnishings, wall decorations, and positions of light sources of the room in no way resembled those of the room containing the touchscreen apparatus.

Additionally, there was a 40 x 32 cm light grey plastic reward box containing one blue bowl identical to the one in the arena apparatus (diameter 8 cm x height 2.5 cm). The bowl was placed in the centre at the short end of the box. The reward box stood in next to the arena apparatus.

Stimuli: The digital stimuli presented on the touchscreen consisted of a 2.5 cm equilateral red triangle presented centrally at the height of the tortoise platform, followed by two 2.5 cm diameter blue circles 10 cm apart and 5 cm above the position of the triangle. All tortoises were able to reach these targets without moving from a central location directly in front of the screen.

The physical stimuli presented during the *arena reversal training* consisted of a black cardboard barrier (43 x 30 cm) showing a red triangle (10 x 9.5 cm) which was positioned in the centre of the arena and of two blue bowls . The bowls (diameter 8 cm x height 2.5 cm) were positioned at one end of the arena at a distance of 50 cm from the starting position and placed 50 cm apart. They contained one piece of food each. The food in one bowl was covered by a perforated, odor- permeable, see-through plastic cover. The food rewards and cover were arranged so that they only became visible to the tortoise when it had approached close to the bowl and thus made a choice.

Procedure

The experiment was run over a period of 33 weeks between 22 December 2010 and 12. August 2011. The animals were tested five days a week between 9 am and 5 pm.

Habituation: The animals were habituated to the apparatus by being placed into the touchscreen box and the test arena individually for 30 minutes. In the touchscreen box food items were freely available in the feeder hole and in the arena food was provided in one blue feeding bowl in the centre of the arena. To ensure that the tortoises were habituated to the sound and vibrations caused by the feeder the tortoises were placed in the touchscreen box and presented with an unchanging white screen while the feeder was operated presenting food in regular intervals. Habituation was considered complete when the animals had eaten readily for three sessions.

Touchscreen pre-training: Pre-training began with an auto-shaping phase during which the tortoises were presented with a photograph of a strawberry which appeared at regular intervals accompanied by a reward. Next the tortoises were manually shaped using a successive approximation procedure. The experimenter manually triggered the feeder in response the tortoises showing ever closer approximations of the desired behaviour of pecking the stimulus on the screen. Once able to peck and initiate the release of a reward by themselves, the tortoises were provided with a number of pre-training phases (table 1), requiring first one peck on each stimulus, then two pecks and then the pecking of different stimuli. The tortoises needed to fulfil the minimum number of trials assigned to each phase and perform reliably for at least three sessions in a row (table 1) before being presented with the training stimuli.

Table 1: Phases of the acquisition of touchscreen operation with regards to stimulus presentation, feeder release mechanism and the response required of the tortoises.

Type of training	Stimulus	feeder activity	response required	min. # of sessions
Auto-shaping	Strawberry	simultaneous with stimulus presentation	take food from feeder	3
Manual shaping	Strawberry	activated by experimenter in response to tortoises' gradual stimulus approach	move towards screen & start pecking	2
Shaping 1	Strawberry	response to pecking	1 peck on stimulus	10
Shaping 2	Strawberry	response to pecking	2 pecks on each stimulus	10
Shaping 3	red triangle and blue circle	response to pecking	2 pecks on stimulus	10
Sequence 1	red triangle and blue circle	response to pecking	1 peck triangle first, then 1 peck on circle	10
Sequence 2	red triangle followed by blue circle	response to pecking	1 peck on triangle followed by 1 peck on circle	10

Touchscreen training: Touchscreen training too place between 04 April and 06 May 2011. The spatial task on the touchscreen started with the presentation of the red triangle. Pecking this was required to start each trial. Once the triangle was pecked it disappeared and the two blue circles appeared. For each tortoise one side, left or right (counterbalanced across individuals), was positive and the other side was negative. If the correct side was pecked both stimuli disappeared and a reward was provided by the feeder, if the incorrect side was chosen the tortoise was given a 3s time out and the trial was repeated until the correct choice was made. Any repeated trial did not count in analysis of correct choices. The criterion for success on this task was a minimum of 200 completed trials with performance being significantly above chance during the last three sessions of 20 trials each.

Arena test: Once a tortoise had successfully completed the *touchscreen training* it was given 20 test trials in the arena apparatus. Tortoises were tested on two consecutive days directly following the last touchscreen training day. They were placed in the arena behind a black barrier showing a red triangle. The trial was started by lifting the black barrier and releasing the tortoise to walk towards one of the blue bowls. During each trial the experimenter stepped out of the tortoises' range of vision immediately after placing it in the arena. This was done to minimize any potential experimenter influence. The trial was ended and the chosen side was recorded when the tortoise had approached within five cm of bowl. Then the tortoises were placed into the reward box where they received a food reward from a blue bowl, irrespective of the side they had chosen in the arena. This reward procedure was designed to minimize unwanted effects of rewarding either or both sides while maintaining the tortoises' motivation to work in a setup they had never before encountered. Between trials the wood shavings covering the arena floor were rearranged to avoid the formation of an odour trail leading in one particular direction.

Arena reversal training: After completion of the *arena test* between 30 May and 11 July 2011 the tortoises received reversal training in the arena apparatus. The side (left or right) which was rewarded during the *touchscreen training* was now unrewarded, and the opposite side was now rewarded. The procedure was identical to that of the *arena test* except that, if the correct bowl was chosen, the tortoise was allowed to approach the bowl and eat a reward from it. If the incorrect bowl was chosen the tortoise was removed from the arena. The criterion for success on this task was the same as for the touchscreen training, a minimum of 200 completed trials with performance being significantly above chance during the last three sessions of 20 trials each.

Touchscreen test: After successful completion of the *arena reversal training* the tortoises' side choice on the touchscreen was tested. They were given 20 test trials on two consecutive days directly following the last day of training. The procedure was identical to that of the *touchscreen training* except that once one circle had been pecked they too disappeared and the tortoise was subjected to a five to ten seconds wait before receiving a food reward from the feeder irrespective of which side they had pecked. The first approach within 0.5 cm of one of the stimulus as recorded on video was analysed rather than the successful pecking of a stimulus. This measure was chosen because this method more closely resembled the analysis of the arena test where the first approach to a bowl was recorded and analysed and the memory of a spatial location rather than the memory of the practicalities of operating the touch screen was of interest.

Arena test 2: After the touch screen test the tortoises were subjected to a second test in the arena which was identical to the first arena test.

Results

Habituation: All four tortoises completed habituation of both the touchscreen and the arena apparatus within three trials. Habituation to the feeder took four trials for Esme and Quinn, 10 trials for Molly and 18 trials for Emily.

Acquisition of touchscreen operation: All four tortoises learnt to successfully operate the touchscreen and to collect rewards from the feeder. *Table 2* shows the trial numbers required by each individual to reach the criterion for moving on to the next training phase.

Table 2: Number of training sessions required by the tortoises for successful acquisition of the various phases of the touchscreen training.

Type of training	response required	min. # of sessions	Esme	Quinn	Molly	Emily
Auto-shaping	take food from feeder	3	3	10	3	12
Manual shaping	move towards screen & start pecking	2	2	2	3	3
Shaping 1	1 peck on stimulus	10	10	10	11	10
Shaping 2	2 pecks on each stimulus	10	10	10	10	10
Shaping 3	2 pecks on stimulus	10	10	10	10	14
Sequence 1	1 peck triangle first, then 1 peck on circle	10	(20*)	-	-	-
Sequence 2	1 peck on triangle followed by 1 peck on circle	10	10	10	33	24

*As Esme, the first to reach this phase of sequence-training, was unable to learn the correct response to this stimulus arrangement, the training procedure was altered and the other tortoises did not engage in this phase of the training.

Touchscreen training: Emily and Molly did not progress to this stage as they stopped working during the sequence 2 stage of training. The reason for this is unknown reasons, as up to this stage they had performed willingly and with levels of success comparable to those of Esme and Quinn. Esme and Quinn, however, reached the criterion of three sessions with above-chance performance within the minimum number of 200 trials (*figure 1*). Above-chance performance of a session was determined by a binomial test with Esme giving 18 ($p < .001$), 19 ($p < .001$) and 15 ($p = .041$) and Quinn 19 ($p < .001$), 17 ($p = .003$), and 20 ($p < .001$) correct responses. Furthermore, a t-test comparing the first three sessions with the last three sessions showed a highly significant effect of learning for both Esme, t(59) = -4.658, p < .001, and Quinn, t(59) = -4.764, p < .001.

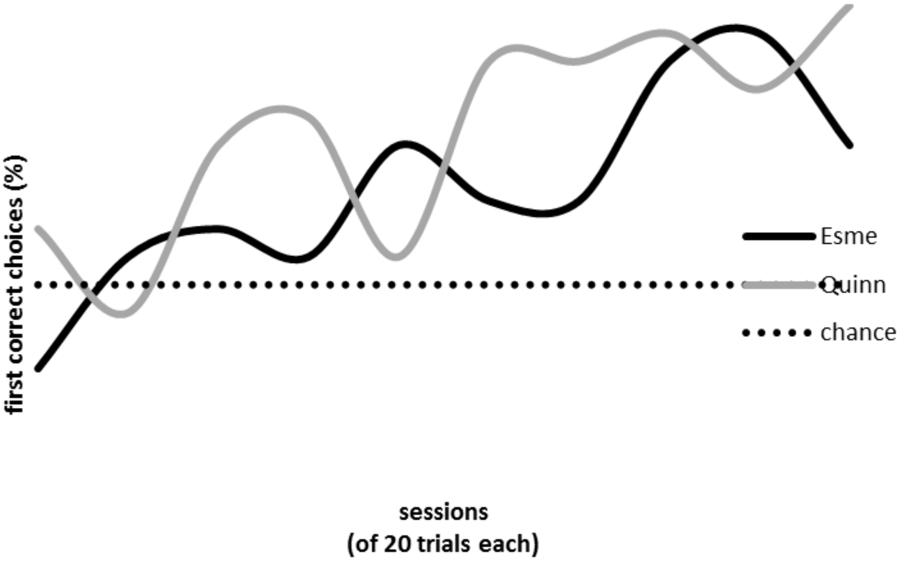

Figure 1: Learning curve of the touchscreen training for Esme and Quinn.

Arena test: A binomial test showed that both Esme (p < .001) and Quinn (p = .012) chose the arena side which was rewarded during touchscreen training significantly more often than would be expected on the basis of chance (*figure 2*).

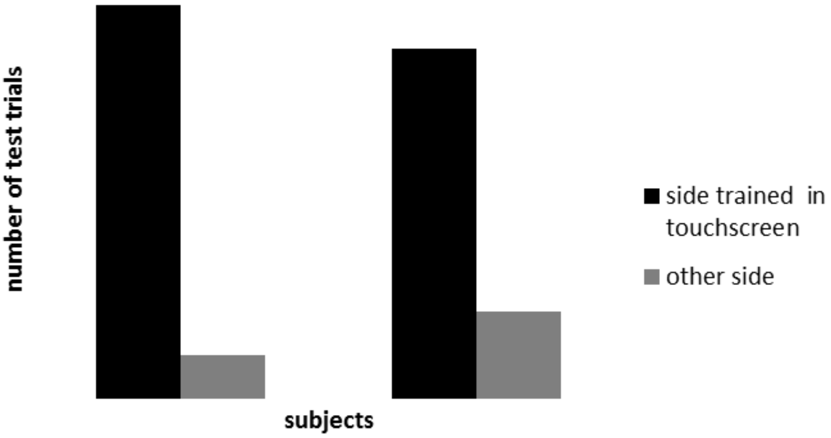

Figure 2: Number of choices made by the tortoises in the arena in the direction rewarded during touchscreen training in comparison to the previously unrewarded side.

Arena reversal training: Esme and Quinn reached the criterion of three sessions with above-chance performance within the minimum number of 200 trials (*figure 3*). Above-chance performance of a session was determined by a binomial test with Esme giving 15 (p = .041), 20 (p < .001) and 15 (p = .041) and Quinn 18 (p < .001), 17 (p = .003), and 18 (p < .001) correct responses. Furthermore, a t-test comparing the first three sessions with the last three sessions showed a highly significant effect of learning for both Esme, t(59) = -4.324, p < .001, and Quinn, t(59) = -5.864 p < .001.

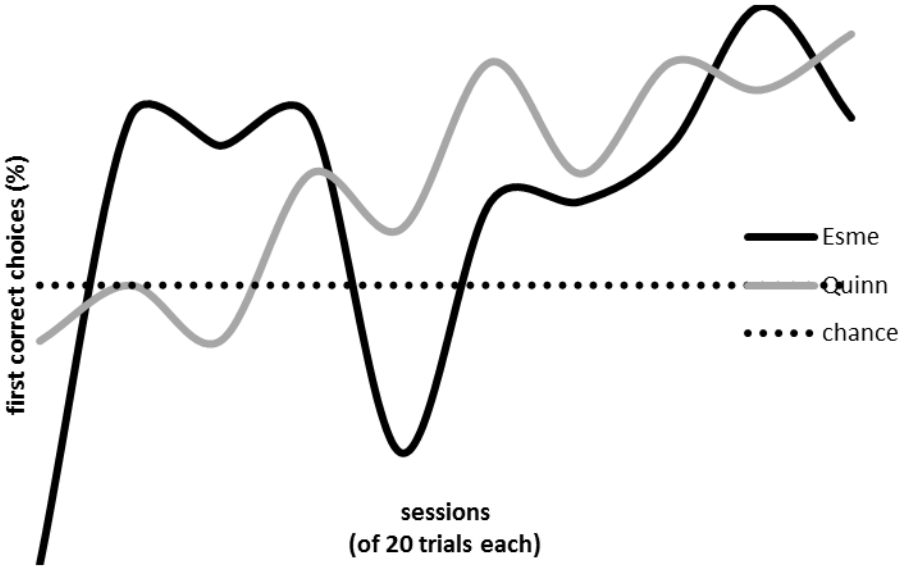

Figure 1: Learning curve of the arena reversal training for Esme and Quinn.

Touchscreen test: A binomial test showed that both Esme (p = .003) and Quinn (p < .001) chose the side on which they were initially trained in the touchscreen significantly more often than chance and significantly less often than the side that they would be expected to have chosen if they had transferred information about the reversal into the touchscreen apparatus arena (*figure 4*).

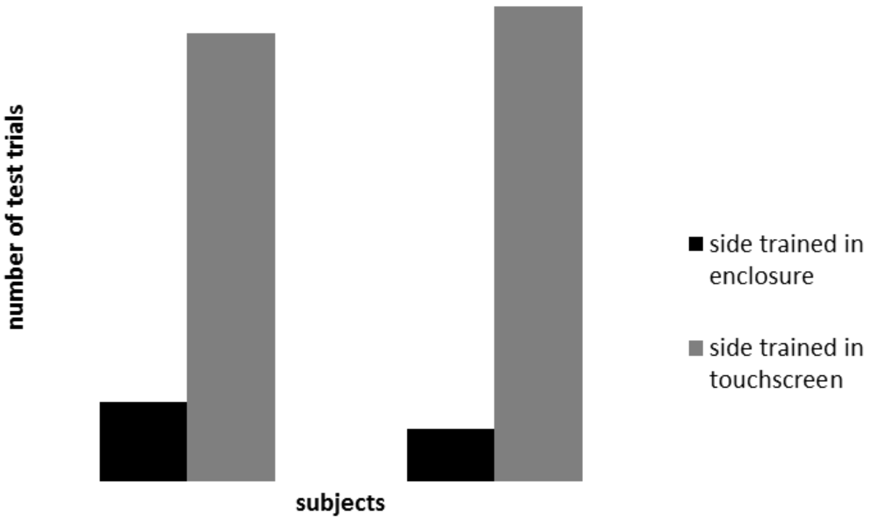

Figure 4: Number of choices made by the tortoises in the touchscreen in the direction rewarded during the reversal training in the arena in comparison to the side rewarded in the earlier touchscreen training.

Arena test 2: A binomial test showed that both Emily (p = .003) and Quinn (p = .041) chose the side which was rewarded during reversal training in the arena significantly more often than the side that had in the past been rewarded in the touch screen (*figure 5*).

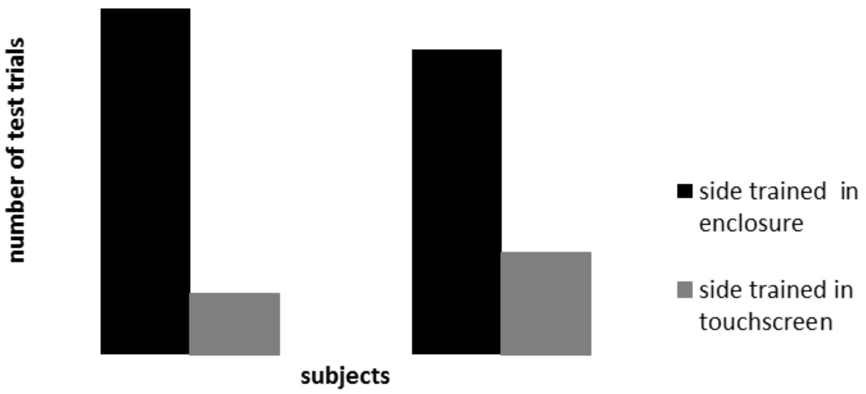

Figure 5: Number of choices made by the tortoises in the arena in the direction rewarded during reversal training in the arena in comparison to the side rewarded in the earlier touchscreen training.

Discussion

The results show that red-footed tortoises are capable of quickly learning to operate a touchscreen. This is the first evidence of such behaviour in reptiles, however it is in line with evidence from Bitterman (1964) showing the terrapins could quickly learn to use a pecking key. It is likely that tasks requiring pecking actions are well within the behavioural repertoire of red-footed tortoise.

Furthermore this experiment reveals that the tortoises were able to transfer learning from the touchscreen to a 3D test arena. After learning that one particular presentation side is positive when two identical stimuli are presented on the touchscreen, the tortoises showed the same side preference when tested in a completely different domain, a 3D arena. As such, the tortoises show an ability comparable to that demonstrated in pigeons (*Columba livia,* Kelly & Spetch, 2004; Kelly, Spetch, & Heth, 1998). The findings suggest that learning acquired in the touchscreen setup might result in broad, generalisable knowledge that can be transferred to a different situation spontaneously and without additional training.

In comparison, when tested in the touchscreen after successfully completing reversal training in the arena, the tortoises did not choose the side which had been rewarded in the arena but the side that had previously been rewarded in the touch screen. This might indicate that learning that has taken place in the arena is less generalisable than learning in the touch screen. However, a different and more parsimonious explanation would be that the tortoises were influenced by their previous experience in the touchscreen setup. To examine this hypothesis, a second side choice test in the arena was run after the touchscreen test. This time the tortoises chose the side which had been rewarded during the arena reversal training. The results show that the tortoises were able to distinguish between the apparatus and the requirements associated with the different setups. The data support the hypothesis that the setup itself is a stronger trigger for position choice than the training provided immediately before the test. However, this does not exclude the possibility that transfer from the arena to the touchscreen would have occurred if no touchscreen training had taken place before.

In addition to showing an ability to distinguish between different choice behaviours that are appropriate in different setups the results suggest that red-footed tortoise maintained long-term memory for spatial strategies. At the time of the touchscreen test the tortoises had not entered the touchscreen setup for over two months during which they were involved in a reversal training in the arena task. Despite this break, and the interfering training, the tortoises performed significantly above chance in the touchscreen test. This suggests that the learning that took place in the touchscreen might not only be broad and generalisable but also reliable and lasting.

The tortoises high performance on the touchscreen tasks clearly shows that experience of the “birds-eye” perspective is not needed to perform well on a task presenting an overview of a test situation. Furthermore, the setup of the touchscreen task was arranged in a way to minimise the amount of movement required from the animals to solve the task. As such success within this setup indicates that motor-feedback produced by walking, at least, is not required for spatial learning in the red-footed tortoise. However, it is still possible that motor-feedback produced by the neck movements might be required.

In conclusion, red-footed tortoises are able to operate a touch screen to learn a simple spatial task. Furthermore, knowledge acquired in the touchscreen can be transferred to a different domain, a 3D arena. However, a specific setup seems to be a stronger trigger for strategy choice than temporal proximity of training. Furthermore, learning which resulted from a simple spatial task acquired on the touch screen seemed to be both generalisable and lasting. This suggests that the touch screen is a viable and valuable method for testing tortoises.

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Chapter 8: Conclusions

Julia Paul

Conclusions

The findings of the present dissertation do not support the notion that reptiles are “sluggish and unintelligent creatures” (Yerkes, 1901, p. 520). On the contrary, the reptiles studied were able to solve all the problems they were presented with in the course of this work, showed a wide range of behavioural flexibility, and demonstrated cognitive abilities comparable to those of mammals and birds.

Chapters 1 and 2 have introduced some specific concepts of studying the cognition of reptiles and have given an overview of the field of reptile spatial cognition. This has been experimentally explored in chapters 3 to 7. The studies described in this thesis attempted to further the knowledge on spatial cognition in reptiles using controlled laboratory experiments. They were concerned with short-distance navigation of two very different reptile species, the red-footed tortoise and the jewelled lizard.

Summary of main findings

Firstly, experimental proof was produced that not just one single red-footed tortoise (Wilkinson et al., 2007, 2009) can solve a radial arm maze but that this achievement lies well within the capabilities of the species (chapter 3). These tortoises have been found to rely on intrinsic cues to an extent greater than previously believed and to favour the use of response strategies to solve a radial-arm maze. In addition, it has been shown that they are able to use a cognitive map-like mechanism to master the maze and that they can use olfactory cues if no visual cues are available. It appears that the determining factor of the strategy preferred in the long-term is the order of acquisition. While it is unclear what exactly determines the initial choice of strategy and what accounts for the individual differences in this choice, it appears that the strategy chosen is the one that remains the favourite of that individual in the future, even when the surroundings change.

Chapter 4 produced evidence that red-footed tortoises are not the only reptilian species that is able to master a radial-arm maze. A jewelled lizard, too, was able to do so, using a turn-by-one arm response strategy similar to that used by two of the tortoises. Given the very distant relation between red-footed tortoises and jewelled lizards, this finding suggests that radial-arm maze navigation is part of the general cognitive capabilities of reptiles. However, more species and individuals need to be tested before more substantial conclusions can be formed.

An initial insight into the extent to which red-footed tortoises are capable of making use of what is provided by their environment to solve complex tasks was discussed in chapter 5. The tortoises, while being a solitary species, were able to socially learn a spatial problem, which they were unable to solve individually. This study provides the first proof of social learning in a non-social reptile, thus questioning the hypothesis that social learning has evolved as an adaptation for group-living. Furthermore, it shows that red-footed tortoises are capable of a variety of behavioural strategies and learning mechanisms when provided with a difficult problem. The fact that encounters between individuals of this species in the wild are very infrequent and are largely restricted to gatherings under fruiting trees (Moskovits & Bjorndal, 1990) suggests that social learning is not a common mechanism in this species. Thus, their ability to learn socially when unable to come to a solution on their own indicates their high levels of behavioural flexibility.

To further test this behavioural flexibility, a comparison between a touchscreen task and a task in a 3D arena was planned. However, to lay the basic groundwork for testing tortoises on a 2D touch screen, it was necessary to investigate their ability to perceive 2D stimuli. Thus, chapter 6 reports a picture-object recognition test that resulted in the demonstration of a solid capability for discriminating 2D photographs. Interestingly, the tortoises had difficulties distinguishing between real objects and photographs of objects of the same kind, suggesting that they perceive them as identical. With this basic knowledge in mind, an across-domain comparison was conducted and described in chapter 7.

The results described in chapter 7 lend further support to the hypothesis that red-footed tortoises possess great behavioural flexibility. The tortoises were able to spontaneously transfer knowledge acquired in the touchscreen to a comparable setup in a ‘real-life’ 3D enclosure. After reversal training in the 3D enclosure the tortoises were tested in both the touchscreen and the 3D enclosure. They showed the ability to distinguish between setups by using the strategy appropriate for each setup rather than only the one they were last trained on. This suggests that the spatial strategies used by red-footed tortoises are chosen deliberately and indicates that they possess a certain level of awareness of their environment and the requirements it poses on their behaviour.

Implications and future direction

The results of the present thesis have shown that red-footed tortoises have great potential as an exemplary study species. They have proven to be flexible, adaptable, very trainable and easy to tame. Furthermore, they have a surprisingly large appetite for a reptile and are eager to work for non-moving food. They have been able to solve tasks in a number of different domains, such as individual spatial orientation, social learning and visual cognition. The behavioural flexibility and individuality, which the tortoises have shown throughout this series of experiments, might be a consequence of their comparatively secure life in captivity as well as in the wild. Their comparative safety might result from having a sturdy shell providing protection against most predators and an environment without regular excessive extremes such as desert temperatures, combined with the comparatively low metabolic requirements of ectothermal animals (Moskovits & Kiester, 1987). All these features of red-footed tortoise ecology are likely to enable them to repeatedly participate in laboratory experiments without experiencing negative stress.

The jewelled lizard has proven a more challenging species. In accordance with its ecology as a vulnerable prey without body armour such as the tortoises are carrying, the jewelled lizard is apt to hide immediately when a possible danger approaches (Díaz, Monasterio, & Salvador, 2006). Thus, even slight noises proved very disruptive to the lizards and led to prolonged periods of complete motionlessness. As such, a laboratory setting within a busy, interruptive university environment might not be the perfect location for testing these sensitive lizards. Additionally, adult jewelled lizards require exceedingly little food and it might be more productive to perfect reptile laboratory setups with an herbivorous lizard that requires greater food before attempting to test this species. Otherwise, it would be recommendable to choose a design which uses hiding holes to reward the animals rather than food.

Unfortunately, the small amount of research in the field of reptile cognition does not allow far reaching conclusions on their comparability with mammals and birds. However, the research that does exist suggests several parallels (Wilkinson & Huber, in press). The spatial navigation behaviour described in the present thesis indicates clear parallels but points towards a shift in emphasis of strategy use. While both rats (Dale, 1986; Dale & Innis, 1986) and tortoises (chapter 3) have been shown to use cognitive-map-like navigation as well as response stereotypy, it appears that the rats usually choose cognitive-map-based navigation as their primary strategy while tortoises regularly choose response stereotypy as their primary strategy. Thus, the difference between strategy choices of the two species might be the preference for one strategy type over the other, rather than the use of different behaviours altogether.

Further research on the spatial cognition of red-footed tortoises is required to fully understand their navigational strategies and to discover what drives the individual differences in their strategy choice. To date, the only predictor of future strategy choice appears to be the strategy first chosen. What determines the first strategy choice is, however, unclear. Are the differences a consequence of the tortoises' flexible ecological make-up reflecting individual preferences or can strategy choice be predicted and manipulated by varying the external cue environment? It is possible that certain cue condition (no cues or cues of particular value) lead to a definite, predictable strategy choice while other, more varied cue conditions leave more room for individual choice and preference. Furthermore there is the possibility that chelonians, or even reptiles generally, display more individual differences regarding strategy choice than commonly observed in most mammals and birds. Davis and Burghardt (2011) found distinct individual differences with respect to strategy choice, individual and observational learning, and learning speed in Florida Red-bellied Cooters (*Pseudemys nelsoni*) solving a non-spatial task. Larger sample sizes, very strictly controlled cues, a greater variety of reptile species, and a broader range of experimental paradigms are needed to determine these open questions.

To understand to what extent and under which conditions chelonians are able to learn socially is still open. Davis and Burghardt (2011) demonstrated stimulus enhancement in Florida Red-bellied Cooters that were able to choose between two colour-coded bottles after having observed one colour providing food to a demonstrator turtle. However, after choosing the correct bottle the turtles were unable to dislodge it as shown by the demonstrator to receive the food reward. While this inability might be caused by an inability to see the exact actions of the demonstrator due the turtles big bodies current evidence does not support the notion that Floria Red-bellied Cooters might be capable of goal emulation or copying (Davis & Burghardt, 2011). Wilkinson, Mandl, Bugnyar, and Huber (2010) have demonstrated that red-footed tortoises follow the gaze of another tortoise in an upward direction. The question that is neither answered by the findings of Davis and Burghardt (2011) or Wilkinson et al. (2010) nor by the results described in chapter 5 is whether this social responsiveness is innate or learnt. As the subjects of all three studies have been kept in group environments for a lengthy period of time before testing their social skills might be acquired. Thus, it is necessary to test tortoises that have been kept individually from hatching on and have thus not had a chance to acquire any knowledge about the advantages of attending to fellow tortoises. Furthermore, tortoises need to be tested in their natural environment to ensure that social attentiveness is not a consequence of life in captivity and the reliance on humans.

The red-footed tortoises had no difficulty learning the operation of a touchscreen, which opens a wide area of possible future research. The use of touchscreen experiments is likely to make reptile research much more comparable to that done with mammals and birds as it to some extent controls for mobility, speed of movements, and long-distance vision. A big advantage of touch screen experiments is the easy manipulation of stimuli. This will particularly improve the testing of tortoise visual cognition. A thorough knowledge of their visual cognition in turn might be the basis for explaining differences in behaviour in other experimental setups such as the radial-arm-maze. The effect of different stimuli types on spatial navigation strategies might be explained by different ways of processing colours, shapes, distance, and geometry. Once the tortoises' visual abilities are tested in depth it will be possible to predict much better which stimuli can be considered good and helpful and which might simply be ignored.

Touchscreen technology is also likely to improve future spatial cognition experiments by creating the opportunity of presenting spatial cues on the screen and testing transferral to real-life set-ups. One such comparison might be to look at differences and similarities of strategy use on a real radial-arm-maze versus a digital one. The overview of the situation provided by the maze presented on the screen might affect strategy choice and might also facilitate learning. However, the feedback received from the physical motion through the maze might be advantageous for maze learning. The basic spatial touchscreen task described in chapter 7 indicates that physical movement is not necessary for learning a simple directional task and transferring it to a different domain. However, whether this holds true for more complex spatial tasks with high working memory involvement is to be tested.

In conclusion, the presented studies on reptile cognition have proven a fruitful and worth-while endeavour that should be continued and extended. For a detailed understanding of the evolution of cognition it is essential to discover the differences and similarities between reptile, mammal, and bird cognition. The limited amount of data in existence to date suggests that cognitive processing of the different amniotic groups might be quite similar. However, it is not yet possible to conclude whether these similarities result from a common ancestry or are the product of a general ability to learn. To reach a level of comparability that allows for a full understanding of the abilities of the amniotes much basic research is still needed, particularly in the field of reptile cognition. Yet, the results presented in the present thesis and elsewhere suggest that this is not only possible but very promising indeed.

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Curriculum Vitae

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EDUCATION

2008 – present	**PhD in Animal Cognition**, University of Vienna, Austria
2010 – 2011	**Certificate in Animal Assisted Therapy**, Veterinary University Vienna, Austria
2005 – 2006	**MSc in Foundations of Clinical Psychology**, Bangor University, UK Overall grade: Distinction
2002 – 2005	**BSc in Psychology**, University of Sunderland, UK Overall grade: First Class Honours, also awarded the 'Academy Prize for Psychology' for the best psychology single honours degree of the class
2004 – 2005	**NCFE Level 3 Certificate in Counselling Skills**, City of Sunderland College, UK
2005	**NCFE Level 1 Certificate in Counselling Children and Adolescents**, City of Sunderland College, UK
2004	**NCFE Level 2 Certificate in Counselling Skills**, University of Sunderland, UK
1991 – 2001	**Abitur** (International Baccalaureate equivalent) in German, Geography, Mathematics and Arts, Eichenschule Scheeßel (high school), Germany

RELEVANT WORK EXPERIENCE

2009 & 2010	**Research Assistant** at University of Vienna, Austria (part time)

Duties: Designing, organising and conducting research for the Cold-Blooded Cognition lab of the Cognitive Biology department, work focused on tortoise and lizard cognition.

RELEVANT WORK EXPERIENCE cont.

2006 – 2008	**Research Assistant** at Bangor University & Universität Heidelberg (collaboration), Bangor, UK (full time) Duties: Designing, organising and conducting psychological research studies with a focus on EEG, eye-tracking and behavioural methods, MSc project supervision, experiment programming, English/German translations
2003	**Research Assistant** at the University of Sunderland, UK (part time) Duties: Conducting structured interviews as part of a study on the tourist industry in the North Tyneside area.

VOLUNTARY WORK

2010 – 2011	**Foster Home** for Hoffnungspfoten (animal rescue organisation), Vienna, Austria
2007	**Dog Walking Volunteer** at RSPCA Bryn-y-Maen Animal Centre, Upper Colwyn Bay, UK
2004 – 2005	**Listening Volunteer** at Samaritans, Sunderland, UK

RESEARCH EXPERIENCE & SKILLS

Reptile navigation	Spatial navigation of the red-footed tortoise and the jewelled lizard Techniques used: behavioural cognitive testing, touchscreen-testing
Tortoise cognition	Social and visual abilities of the red-footed tortoise Techniques used: behavioural cognitive testing, touchscreen-testing
Alcohol addiction	Cognitive dis-inhibition underlying addiction Techniques used: EEG, computerised cognitive tests, questionnaires
Art & music	The EEG signature of music and art perception and appreciation Technique used: EEG, Eye-tracking, questionnaires
Schizotypy	Social cognition, executive functions, and variability in schizotypy Technique used: computerised cognitive tests, questionnaires
Memory & aging	Electrophysiological correlates of music memory in Alzheimer's disease: *MSc thesis, graded A** Technique used: EEG and ERP, questionnaires

Attractiveness	Healthy, successful, and altruistic: How sexy is that? An evolutionary explanation: *BSc thesis, graded 1st class* Technique used: questionnaires

ADDITIONAL INFORMATION

Languages	English (perfectly fluent, oral and written) German (mother tongue) Spanish (basic)
Software	MS Office, SPSS, E-Prime, Photoshop, EyeTrace, BESA, Brain Vision Analyser, Visual Site Designer
Driving licence	Cars and motorcycles, clean, car available
Interests	Animal fostering; dog walking; my pets; swimming; mountain walking; playing the flute & piccolo; reading; visiting theatre, musical & opera performances.

LIST OF PUBLICATIONS

Academic papers

Mueller-Paul, J., Wilkinson, A., Aust, U., Steurer, M., Hall, G., & Huber, L. (in prep.). Red-footed tortoises (*Geochelone carbonaria*) are capable of transferral between the touch screen and a physical arena.

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Invited talk	**Mueller, J.** (2010). Räumliche Orientierung von Reptilian (Spatial navigation in reptiles). *Nationalhistorisches Museum Wien*, Vienna, Austria.
Poster	**Mueller, J.**, Wilkinson, A., Hall, G., & Huber, L. (2010). Response-based navigation in the jewelled lizard. *Konrad Lorenz Forschungsstelle anniversary symposium*, Vienna, Austria.
Talk	**Mueller, J.** (2009) Spatial learning in reptiles: Are tortoises better than lizards? *CogBio Seminar*, University of Vienna, Vienna, Austria.
Talk	**Mueller, J.**, Wilkinson, A., Künstner, K. & Huber L. (2009) What does a reptile see in a picture? Picture-object recognition in the red-footed tortoise (Geochelone carbonaria). *ESF conference*, Budapest, Hungary.
Talk	Wilkinson, A., Kuenstner K., **Mueller, J.**, & Huber, L. (2009). Social learning in a non-social reptile: Can a tortoise (Geochelone carbonaria) learn from the actions of a conspecific? *ESF conference*, Budapest, Hungary.