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The underestimated giants: operant conditioning, visual discrimination and long-term memory in giant tortoises

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Abstract

Relatively little is known about cognition in turtles, and most studies have focused on aquatic animals. Almost nothing is known about the giant land tortoises. These are visual animals that travel large distances in the wild, interact with each other and with their environment, and live extremely long lives. Here, we show that Galapagos and Seychelle tortoises, housed in a zoo environment, readily underwent operant conditioning and we provide evidence that they learned faster when trained in the presence of a group rather than individually. The animals readily learned to distinguish colors in a two-choice discrimination task. However, since each animal was assigned its own individual colour for this task, the presence of the group had no obvious effect on the speed of learning. When tested 95 days after the initial training, all animals remembered the operant task. When tested in the discrimination task, most animals relearned the task up to three times faster than naïve animals. Remarkably, animals that were tested 9 years after the initial training still retained the operant conditioning. As animals remembered the operant task, but needed to relearn the discrimination task constitutes the first evidence for a differentiation between implicit and explicit memory in tortoises. Our study is a first step towards a wider appreciation of the cognitive abilities of these unique animals.

Introduction

Giant tortoises have been described as "living rocks" that are slow in both movement and cognitive abilities (van Denburgh 1914). Although there were some early studies on turtle behavior (Yerkes 1901), the scientific and non-scientific communities have generally considered giant tortoises,

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and many other turtles, to be behaviorally inflexible. But even early descriptions of tortoises suggest otherwise. Van Denburgh (1914), who collected early accounts on the giant tortoises of the Galapagos Archipelago and their near extinction, reported: "The tortoises do a great deal of apparently unnecessary traveling; and, though slow, are so persistent in their journeys that they cover several miles a day." In fact, Darwin (1878) observed that when encountered in their natural habitats, they often traverse considerable distances from sleeping areas to feeding grounds, water sources or mud patches. An early explorer even described the ease of training tortoises held on ships to remain in a specific area; in a procedure we would now call negative reinforcement training (Delano 1817).

Until recently, reptiles were in general often regarded as reflex machines, incapable of more complex behaviors, and were thus reduced to a footnote in the evolution of learning and intelligence (Harless and Morlock 1979). In the mid-1960s several researchers began revisiting the study of learning phenomena across different taxa in an effort to measure performance differences (see e.g. Bitterman 1965, 1975). Several years later, Burghardt (1977)

wrote his review on learning in reptiles. In recent years, more work on turtles and other reptiles has emerged and we are finally starting to fill in the blanks on the evolution of cognitive behaviors (see Matsubara et al. 2017 for a review).

Positive Reinforcement Training (PRT) is a form of operant conditioning in which an animal is rewarded for responding to a stimulus in a non-reflexive manner, thus encouraging the animal to reproduce the same response when presented with the same stimulus. It is an important aspect of environmental enrichment in many species of animals kept in zoos (see Desmond and Laule 1998 for review), where it is often performed in a strictly goal-directed manner, e.g.: to guide an animal to a certain part of the enclosure or to assume certain body postures to facilitate health checks (Fernandes and Timberlake 2008; Grandin et al. 1995; Phillips et al. 1998). Studies have described positive reinforcement training (Gaalema and Benboe 2008) and operant conditioning (Weiss and Wilson 2003) of Aldabra giant tortoises (*Aldabrachelys gigantea*) in zoos. These works, which focused on enrichment and animal handling, demonstrated the ability of the animals to learn. Because operant conditioning is the basis for many discrimination learning tests, it is quite valuable for comparative studies on a wide variety of non-model animals, work that in many cases would be unfeasible in a non-zoo setting. Results of such experiments can help elucidate the evolutionary origins of various cognitive abilities.

Recently, studies by Wilkinson et al. (2010a, b) and Davis and Burghardt (2011) showed, for the first time, that turtles are capable of social learning, whereby a naïve animal can acquire information from a knowledgeable one, while avoiding the costs of learning on its own (Heyes 1994). Social learning has been shown in mammals (e.g.: Hoppitt and Laland 2008; Huber et al. 2009), birds (Huber et al. 2009; Slagsvold and Wiebe 2011), reptiles (Davis and Burghardt 2011; Kis et al. 2015; Wilkinson et al. 2010a, b), bony fish (Brown et al. 2011; Laland et al. 2003), cartilaginous fish (Thonhauser et al. 2013) and even in invertebrates (Fiorito and Scotto 1992; Leadbeater and Chittka 2007).

Although neither Aldabra nor Galapagos (*Chelonoides nigra* and related species) giant tortoises have been described as particularly social animals, in the wild, they do aggregate in high densities (van Denburgh 1914; Grubb 1971). Field work on Aldabra tortoises revealed a non-sexual social behavior termed 'nosing', in which an individual approaches another tortoise and noses the head or neck, holding the position for up to several minutes (Grubb 1971). No explanation for this behavior has been suggested. In the Galapagos Islands, tortoises sleep, graze and migrate in groups, and agonistic dominance altercations have been observed (van Denburgh 1914). It thus seems reasonable to speculate that social learning may be an important feature of the tortoise behavioral repertoire.

In the present study, we focus on operant learning tasks in two species of giant tortoise: Aldabra tortoises and Galapagos tortoises (*Chelonoidis* cf. *nigra*). We predicted that tortoises would acquire a PRT task faster when trained in a group as opposed to individually. We created two different conditions by dividing the task into two stages: first, a simple go to target, and second, a task that requires memory of a specific target. For the first, animals performed a task that is similar to that reported previously (Davis and Burghardt 2011; Soldati et al. 2017). For the second, we asked each tortoise to remember a specific color, with a different target color assigned to each individual. This allowed us to determine whether a tortoise just remembered the task in general, or whether it remembered the specific target color. We speculate that the stage 2 task—"go to target"—is related to implicit memory, while the more specific recall of the correct target color is mediated by explicit memory (Schacter 1987).

In addition to the general ability of tortoises to be trained in an operant task, we also investigated their ability to learn a simple, two alternatives, forced choice test. We tested them 3 months after the initial experiment to determine their long-term memory of both the general procedure task and the color discrimination task (Davis and Burghardt 2007). Remarkably, some individuals seemed to remember the tasks 9 years after the initial training sessions.

Method

Animals

Vienna zoo

Three adult Aldabra tortoises (two male, one female; aprrox. 100, 80 and 60 years old) (wild caught), and four sub-adult (two male, two female; all 14 years old) Galapagos tortoises (bred in the Zürich zoo), that were housed together in a mixed species group. During the period of the experiment, due to renovation, the tortoises were situated in a temporary enclosure. The enclosure consisted of two connected rooms (dimensions), one of which included windows to the visitors' area. The animals could move freely between the rooms throughout the day, and food was available in both rooms. For training, the rooms were physically and visually separated by a sliding gate. During each training session, only one animal was present with the experimenter in the training arena. One male adult Aldabra tortoise stopped participating during the first stage of the study and was, therefore, not included in color discrimination training.

Zürich zoo

Galapagos tortoises—two adult (one male, one female; 50–60 years old) (of unknown origin), and two juvenile (unsexed; 3 years old) Galapagos tortoises (bred in the Zürich zoo)—were housed together in a single species exhibit. One additional adult female tortoise shared the enclosure but did not participate in the experiments and is not included in the analysis.

Aldabra tortoises—Four adult (two male, two female; age unknown) and two juveniles (unsexed; < 3 years old) (all of unknown origin)—were housed together in a single species exhibit, part of a large indoor enclosure, the Masoala hall. One additional adult female tortoise shared the enclosure but did not participate in the experiments and is not included in the analysis.

Pre-training

Based on communication with the zoo-keeping staff, food items were selected out of the normal feeding diet to be used as positive reinforcement. The animals were fed their normal meal immediately after all animals in the group finished the daily training. The items were then presented to the individuals or the group for 5 days (with ten repetitions per day). Each item was presented at the end of a dowel to the area of the mouth. If an animal refused a food item, the item was not chosen as reinforcement for that individual, and the trial was repeated until several preferred food items per animal were established (this was done to ensure that if one item would be not available another could be chosen instead). Food items selected as reinforcements were then removed from the regular feeding schedule and provided as reward during the training, and at the end of the training day. This procedure ensured that the food items were consistently attractive to the animals and allowed for a period of familiarization between experimenters and animals.

Experimental protocol

Stage 1: bite target

Animals were presented with a colored dog toy attached to the end of a dowel (Fig. 1a). Early research demonstrated a preference for the color orange in Galapagos tortoises (Evans and Quaranta 1949); we, therefore, chose to exclude the color orange from target colors (bright blue target for Vienna zoo Aldabra and Galapagos tortoises; bright blue for Zürich zoo adult Galapagos tortoises; pale green for juvenile Galapagos tortoises; pale yellow for Zürich zoo Aldabra tortoises).

At the beginning of training, using a shaping procedure, the target (ball) was touched to the nose of the tortoise to draw its attention to the stimulus. A food reinforcement, which was kept hidden, was then delivered directly to the mouth (at the end of a separate dowel). To prevent "eavesdropping" by other tortoises (in the individual training), we chose to make reinforcement immediate and without an auditory bridge (such as a clicker), in both individual and in-group training sessions. After successful shaping, tortoises began to bite the target voluntarily, without the need to touch the target to the nose contact, to receive the reinforcement (Fig. 1a, c); these were considered as correct trials.

Each trial lasted up to 3 min, or until the target was voluntarily bitten, with an intertrial interval of 1 min, or until the food reinforcement was consumed. If no voluntary contact was made with the target at the end of the 3 min, the target was touched to the nose and reinforcement given, followed by a 3-min intertrial interval. Biting the dowel instead of the target resulted in an aborted trial and was followed by a 3-min intertrial interval. Each individual was presented with the target until it performed ten correct trials on a given day (repeating a trial for each incorrect trial).

Stage 1 was completed when the animal voluntarily bit the target in ten out of ten trials in a single day, with no aborted or incorrect trials. For statistical comparisons, we used the last trial in which a mistake was made as a measure of learning.

Stage 2: go to target

The target was presented at a distance of 1.5–2 meters (for adult and sub-adult), or 0.8–1 m (for juveniles) from the tortoise (Fig. 1b, d, supplementary video SV1). In each trial, the target was presented at full distance; if within a minute, no movement was made the target was brought to half distance and the process repeated. Intertrial interval was 1 min or until the food reinforcement was consumed. If no movement was made within 3 min, the trial ended, and was followed by an intertrial interval of 3 min. No movement in three consecutive trials ended the training for the day. Biting the dowel instead of the target resulted in an aborted trial and was followed by a 3-min intertrial interval. Each individual performed ten correct (full or partial distance) trials a day (repeating a trial for each incorrect trial).

Criterion for learning the task was completion of ten consecutive full distance "go to target" trials during 1 training day, with no partial distance or incorrect trials. For statistical comparison, we used the last trial in which a mistake was made as a measure of learning.

In-group vs. individual training in Stages 1 and 2

Individual training (Vienna zoo)—before each session, a single animal was separated from the group and led to the experimental arena. In the experimental arena, the animal

Fig. 1 a Target blue target presented to Galapagos tortoise at the Vienna zoo (Stage 1 bite target individual training). **b** Galapagos tortoise at the Vienna zoo walking toward the target blue target (Stage 2 go-to target, individual training). **c** Target yellow (ball) presented to Aldabra tortoise at the Zurich zoo (Stage 1 bite target in-group training). **d** Adult Galapagos tortoise at the Zurich zoo approaching the target (Stage 2 go-to target, in-group training). **e** Galapagos tortoise was trained individually, and at the end of the training led back to the group. After the completion of all individual at the Vienna zoo approaching the two colored targets, one colour is correct and the other is a distractor (Stage 3 colour discrimination learning, individual training). **f** Galapagos tortoise at the Zurich zoo approaching the two colored targets, one colour is correct and the other is a distractor (Stage 3 colour discrimination learning, in-group training). White arrows (**c**, **d**, **f**) mark conspecifics (color figure online)

training sessions, the gate splitting the enclosure was opened.

In-group training (Zürich zoo)—neither of the single species exhibits had a physically or visually separated experimental arena. Therefore, when each animal was trained, it was in full view of conspecifics, and was allowed to interact with conspecifics during training. However, animals not being trained were not required to interact or attend to the training and were free to move around the enclosure. Training proceeded as above. For in-group training, the experimenter focused on each individual animal in turn until every animal completed ten correct trials per day. Training of the smaller, juvenile Galapagos tortoises was performed with targets (balls) that were smaller in diameter than those used for adult Galapagos and all Aldabra tortoises.

Training sessions were videotaped, and videos were analyzed using Noldus Observer 10 software.

All experimental sessions in both zoos were conducted by the same experimenters. Training and filming were alternated randomly between experimenters daily.

Stage 3: color discrimination

After completing stage 2 of the experiments, ten of the animals (four Galapagos tortoises from Zurich, four Galapagos and two Aldabra tortoises from Vienna) continued to stage 3. In stage 3, we used an operant conditioning technique commonly called the two-alternative, forced-choice test (Schatz 2011). Using this method, the subject is presented with two stimuli and is reinforced with a food reward when choosing the "correct" stimulus. To prevent the influence of stimulus location on choice, the positions of the stimuli are randomly determined for each trial. The target and the distractor were rubber dog toys similar to the ones used in stage 2. Target and distractor were identical in all but color. To avoid clues to the animals, the experimenter did not look at the target or distractor during the procedure (supplementary video SV2). Due to the size constrains of the indoor enclosure, tortoises in Vienna were visually shielded from each other; the topography of the outdoor area in the Zürich zoo did not allow us to test each animal out of sight of the others. Each tortoise was assigned a different color target which remained unchanged throughout the experiment. During each trial, the target, and an identical but different color distractor target, was presented at the ends of two connected dowels (Fig. 1e,

f). The targets were presented 1.5–2 m away from the animal, and the right or left position of the targets was changed between trials following a restricted randomness sequence (Fellows 1967). Trials were conducted as in stage 2, except that now the animal had to choose to approach only one of the two targets. After the last incorrect choice, the animals were tested for 1 additional day on the same color combination and then moved on to a different color distractor target.

Long-term memory

95 days after completion of stage 3, the animals at Vienna zoo ($n = 6$) were tested again to assess their long-term memory.

Nine years after the testing of long-term memory, the three adult tortoises still available in the Vienna zoo were tested again.

Statistics

Due to the small sample size, data were analyzed using non-parametric statistics. Performances, as measured by last trial in which a mistake was made, of the different species within the two training conditions (individual and in-group) and between the two training conditions were compared using Mann–Whitney U tests. To test the difference in learning speed between stage 3 and long-term memory, a Wilcoxon test was used.

Results

Stage 1 training

All animals in both groups learned to bite the target for food reinforcement (Tables 1, 2). Individually trained, Vienna zoo Galapagos tortoises required 15–77 trials to reach criterion, with a median of 17.5 ($n = 4$). Individually trained, Vienna zoo Aldabra tortoises required 55 and 50 trials ($n = 2$) (Fig. 1a). Individually trained Galapagos tortoises and Aldabra tortoises showed no significant difference between species in the number of trials to reach the

Table 1 Individual performance of animals to reach criterion at the Vienna zoo mixed species exhibit

	Aldb1	Aldb2	Galp1	Galp2	Galp3	Galp4
Bite target	55	50	20	15	15	77
Go to target	60	56	40	54	40	20
Sex	♂	♀	♂	♂	♀	♀
Age group	Adult	Adult	Sub adult	Sub adult	Sub adult	Sub adult

Animals were individually trained in a visually separated part of the enclosure (*Aldb* Aldabra tortoise, *Galp* Galapagos tortoise)

Table 2 Individual performance of animals to reach criterion at the Zürich zoo Galapagos and Masoala Hall exhibits

	Aldb1	Aldb2	Aldb3	Aldb4	Aldb5	Aldb6	Galp1	Galp2	Galp3	Galp4
Bite target	6	3	3	2	2	9	3	5	2	3
Go to target	10	11	11	10	10	26	12	16	17	18
Sex	♂	♂	♀	♀	Unsexed	Unsexed	♂	♀	Unsexed	Unsexed
Age group	Adult	Adult	Adult	Adult	Juvenile	Juvenile	Adult	Adult	Juvenile	Juvenile

Animals were not separated from the group for training (*Aldb* Aldabra tortoise, *Galp* Galapagos tortoise)

criterion (Mann–Whitney $U = 2$, $N_1 = 4$, $N_2 = 2$, $P = \text{NS}$) and are, therefore, analyzed as one group.

In-group trained, Zürich zoo Galapagos tortoises required 3–5 trials to reach criterion, with a median of 3 ($n = 4$). In-group trained, Zürich zoo Aldabra tortoises required 2–9 trials to reach criterion, with a median of 3 ($n = 6$) (Fig. 1c). In-group trained animals in single-species exhibits showed no significant difference between species in the number of trials to reach criterion (Mann–Whitney $U = 11$, $n_1 = 4$, $n_2 = 6$, $P = \text{NS}$) and are, therefore, analyzed as one group. However, individually trained animals took significantly longer to learn the task than animals trained in-group (Mann–Whitney $U = 0.000$, $n_1 = 6$, $n_2 = 10$, $P < 0.005$) (Fig. 2a).

Stage 2 training

Galapagos tortoises required 20–54 trials to reach criterion with a median of 40 ($n = 4$). Individually trained Aldabra tortoises required 60 and 56 trials to reach criterion ($n = 2$) (Fig. 1b). Here as well, Galapagos tortoises and Aldabra tortoises showed no significant difference between species in the number of trials to reach the criterion (Mann–Whitney

$U = 0$, $n_1 = 4$, $n_2 = 2$, $P = \text{NS}$) and are, therefore, analyzed as one group. Aldabra tortoises trained in-group reached criterion in 10–26 trials with a median of 10.5 ($n = 6$). Galapagos tortoises trained in-group reached criterion in 12–18 trials with a median of 16.5 ($n = 4$) (Fig. 1d). Video analysis showed that both Aldabra and Galapagos tortoises trained in-group exhibited no aggressive interactions between conspecifics during training. The two species trained in-group did not show differences in how long it took to acquire the task (Mann–Whitney $U = 20$, $n_1 = 4$, $n_2 = 6$, $P = \text{NS}$) and are, therefore, analyzed as one group. Overall, tortoises trained in-group ($n = 10$), reached criterion significantly faster than individually trained tortoises ($n = 6$) (Mann–Whitney $U = 1$, $n_1 = 6$, $n_2 = 10$, $P < 0.005$) (Fig. 2b).
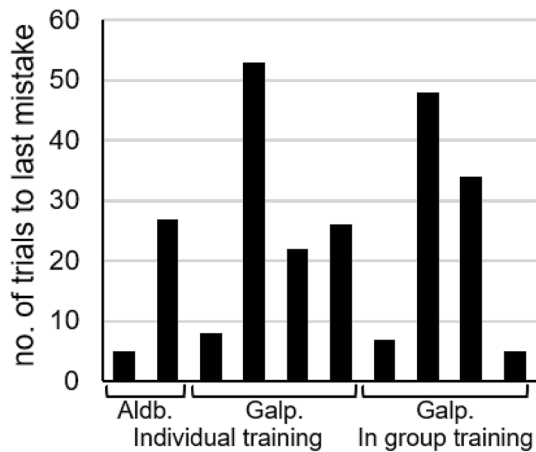
Stage 3

Individually trained Galapagos tortoises required 8–53 trials to reach criterion with a median of 24.5 ($n = 4$) (Figs. 1e and 3). Individually trained Aldabra tortoises required 5 and 27 trials to reach criterion. Four Galapagos tortoises ($n = 4$) trained in-group reached criterion in 5–48
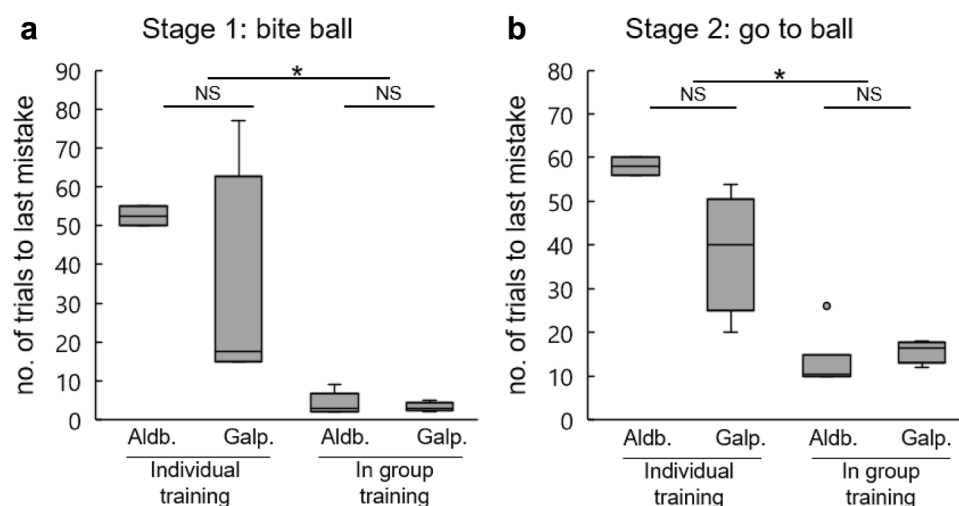

Fig. 2 a Performance in stage 1—bite target: In-group trained Aldabra ($n = 6$) and Galapagos ($n = 4$) tortoises (Zurich zoo) reached criterion significantly faster than individually trained Aldabra ($n = 2$) and Galapagos ($n = 4$) tortoises (Vienna zoo). (Mann–Whitney $U = 0.000$, $n_1 = 6$, $n_2 = 10$, $P < 0.005$). **b** Performance in stage 2—

go-to target: In-group trained Aldabra ($n = 6$) and Galapagos ($n = 4$) tortoises (Zurich zoo) reached criterion significantly faster than individually trained Aldabra ($n = 2$) and Galapagos ($n = 4$) tortoises (Vienna zoo).(Mann–Whitney $U = 1$, $n_1 = 6$, $n_2 = 10$, $P < 0.005$). *Aldb* Aldabra tortoise, *Galp* Galapagos tortoise

Fig. 3 Performance in stage 3—colour discrimination: there was no significant difference in the number of trials to reach criterion for learning between individually trained tortoises (2 Aldabra and 4 Galapagos), and in-group trained Galapagos ($n=4$) tortoises (Mann–Whitney $U=11.5$, $n_1=6$, $n_2=4$, $P=$NS). *Aldb* Aldabra tortoise, *Galp* Galapagos tortoise)
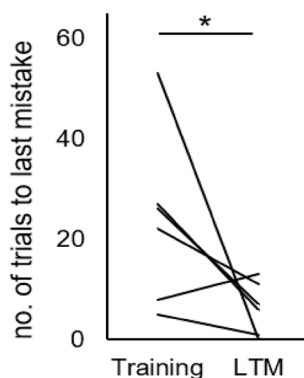
Fig. 4 Number of trials to reach criterion for learning in the original training, and in the long-term memory (LTM) training. Five of the six tortoises (2 Aldabra and 3 Galapagos) required less trials to reach criterion than in the original training

trials, with a median of 20.5 (Figs. 1f and 3). There was no significant difference in learning speed in the two different locations (Mann–Whitney $U=11.5$, $n_1=6$, $n_2=4$, $P=$NS) (Fig. 3). Once the criterion was reached, additional presentation of distractor targets did not affect the performance of the animals.

Long-term recall of training

All animals immediately approached the targets for a reward just as they had in previous trials. None of the animals immediately recalled the specific color of target it was trained to. However, number of trials to last mistake was significantly lower in five of the six animals tested

95 days after initial testing (Wilcoxon, $n=5$, $Z=-2.023$, $P=0.043$; 2 Aldabra and 3 Galapagos) (Fig. 4). One subject (Galapagos) took longer in the recall training then in the initial training.

Nine-year long-term recall

Nine years after the last memory recall training, three Aldabra tortoises in the Vienna Zoo (two males, one female) were still available, and were tested in stage 1 and stage 2 tasks. During the original training, Aldb1 (Table 1) was the dominant male, the non-dominant male (not listed in the table) was mostly unresponsive and unwilling to participate in training and therefore did not progress past stage 1. In the long interval between tests, the dominance between the two males shifted. Although the previously dominant, Aldb1, immediately responded to stage 1, he only completed three stage 2 trials before becoming unresponsive and hiding. In contrast, the previously non-dominant male showed immediate recall of the stage 1 task and completed several stage 2 trials. The female tortoise, Aldb 2 (Table 1), showed immediate recall of both stage 1 and stage 2 tasks but stopped responding after several trials. All three animals immediately responded correctly to bite the target task, and all completed several go-to-target trials, showing recall of the tasks learned in the original experiment and recalled in the long-term memory test, 9 years earlier.

Discussion

All tortoises in this study learned to perform the operant task of biting a novel rubber target for a food reward (Stage 1 training), only one male tortoise in Vienna did not pass the Stage 2—go to target. Like a previous study by El'darov and Sikharulidze (1968), where tortoises were trained to bite a mouthpiece for a food reward, in our experiment, we chose biting as the reinforced behavior. However, the speed of acquisition by our animals was faster than in the previous study. This may be attributed to the fact that our experiments did not use a skinner-box like apparatus. Training in both zoos took place in the home enclosures, in the presence of a familiar human experimenter, avoiding neophobia. This presumably led to the faster speed of learning to use this behavior for a reward.

In both stages of training, in-group trained tortoises acquired the task faster than those trained individually, while there was no difference in the subsequent discrimination learning experiment (Thorndike 1911). In fact, the tortoises trained in-group with conspecifics performed much faster in this task than any Chelonian species previously studied (for reviews, see Burghardt 1977; Weiss and Wilson 2003).

There were no significant differences in performance of both Zurich zoo species groups, thus suggesting that the color of the target had no significant effect on learning. Additionally, there was no significant difference in performance between the two species in both Vienna and Zurich zoo.

Animals trained in-group in the Zürich zoo differed from those trained individually in Vienna both by geographic location and by separation of individuals in the enclosure during training. The differences in enclosures, however, could not account for the overall in-group speed of acquisition, as the two Zürich zoo groups did not share an enclosure yet were not significantly different in performance. This suggests that the presence of conspecifics in training, both in stage 1 and stage 2, enhanced the speed of learning in these groups despite these differences. All the tortoises in our study were fast learners, and those allowed to observe and interact with conspecifics during training learned significantly faster.

Thus, we now provide the first evidence for social learning in Galapagos and Aldabra tortoises. Social learning, even in its most basic forms, is an important tool that allows an animal to gain knowledge without the cost of individual experience. Even though giant tortoises may not form the complex social societies found in other species, they do, in the wild, share resources. It seems likely that they share information about those important resources, drawing attention to a location by directing a gaze and watching the consumption of food or water.

Several types of social learning might account for these results. Studies by Davis and Burghardt (2011) and Wilkinson et al. (2010a, b), showed that turtles were able to use cues provided by trained conspecifics to learn socially. It is likely that stimulus enhancement or perhaps social facilitation affected learning in our experiments. In these circumstances, an observing tortoise would be attracted to the target by seeing a conspecific interacting with it. In addition, since observer animals saw their group-members consume their food rewards during training, it is likely that observational conditioning influenced the social learning process. However, further experiments will be needed to fully reveal the identity and extent of social mechanisms guiding this process.

Following the initial operant training, ten tortoises were trained in a color discrimination learning task. As in the initial training, Vienna zoo tortoises were trained individually (each tortoise was still assigned a unique color target). Among the in-group trained Zurich zoo tortoises only the Galapagos tortoises were trained in the color discrimination task. Performance of all animals was fast and comparable to other vertebrates (Thorpe 1963). Unlike the original training, there was no significant difference in learning speed between the in-group and the individually trained animals. While in the original in-group training, tortoises observed conspecifics responding to the same target presented to them, in the color discrimination trials every individual was assigned a different colored target. Thus, observing the trials of a conspecific is noninformative; therefore, there would no longer be any benefit from in-group training.

We divided the training into two levels of task, the first responding to a general operant task—go to target, the second responding to an individually assigned colored target—go to correct of two target options. With this distinction between tasks, we could test the difference between implicit memory "go to target" and explicit memory "go to a specific target". During the long-term recall experiment, all animals immediately remembered to go to a target for a reward. When testing the memory of the correct color target, all animals needed to be retrained, however, five of the six reached criterion significantly faster than in the original training. This gives a first hint about the presence of both implicit and explicit memory in a tortoise (Burghardt 1977; Matsubara et al. 2017; Schacter 1987; Wilkinson and Huber 2012).

In an attempt to test ultra-long-term memory, we retested the 3 Aldabra tortoises that were originally individually trained in the Vienna zoo. All three tortoises immediately responded to the operant task. However, the male and female that completed the original color discrimination task were too unresponsive to continue with to the color discrimination test. Both responded correctly to the presentation of the target but did not consistently walk the full distance to it. There are several possible reasons for this lack of participation, among which are the shift in dominance between the two male tortoises and the inclusion of all the reward foods in the normal daily diet. Unfortunately, the now responsive male tortoise 10 years earlier was subdominant and unresponsive and, therefore, never trained to a specific color target. Thus, we can say that while the task "go to target" was remembered fully by one subject, and perhaps also by the other two, we could not test whether an individual target would have been remembered.

Our results highlight flexibility in learning in tortoises and support growing evidence of the significance of social interaction and social learning in reptiles. Comparative study of a variety of reptile species, likely including zoo-based research, will allow for a more thorough understanding of the ecology and evolution of learning in reptiles and processes shaping social learning in all vertebrates.

Compliance with ethical standards

Conflict of interest T Gutnick has received an honorarium from Tiergarten Schönbrunn supported by donation from H.H. Lederer.

Ethical approval All applicable international, national and institutional guidelines for care and use of animals were followed.

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