Lateralisation shapes spatial learning in lizards

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Running title: Spatial learning and lateralisation in *P. muralis*

Abstract

Spatial memory is fundamental cognitive process that allows animals to navigate and interact with their environment effectively. While extensively studied in mammals and birds, the mechanisms underlying spatial cognition in reptiles remain less understood. In this study, we investigated spatial learning and the potential influence of behavioural lateralisation in the common wall lizard (*Podarcis muralis*). Using a T-maze, we examined whether lizards could develop short-term spatial memory and whether lateralisation affected their navigation. Experimental lizards received three days of training in the maze without reinforcement, while control lizards had no prior experience. We found that trained lizards rapidly learnt to navigate, reaching a goal shelter faster and more reliably than controls. Additionally, only seven out of twenty individuals exhibited lateralised turning behaviour. Nevertheless, strongly lateralised individuals took longer to reach the goal during training, but this did not necessarily impair performance once the route had been learned. These findings align with research in other taxa, where lateralised individuals often show context-dependent differences in performance, sometimes outperforming non-lateralised individuals under stress. This study contributes to a broader understanding of cognitive evolution across vertebrates and emphasizes the importance of reptiles as models for comparative cognition research.

Keywords*:* Behavioural lateralisation, Brain lateralisation, Spatial cognition, Reptile, Squamate

Introduction

Spatial memory and place learning are fundamental cognitive processes that enable individuals to navigate, recognize, and remember their environment through mental representations, such as cognitive maps (Healy, 1998). These abilities are essential for behaviours critical to survival and reproduction, such as foraging, avoiding predators, finding mates, and selecting habitats (Gautestad, 2011; Rosati et al., 2014; Heathcote et al., 2023). Different taxa have evolved different strategies to encode, process, and retrieve spatial information, depending on ecological pressures and lifestyle.

Among invertebrates, honeybees (*Apis mellifera*) rely on a combination of visual landmarks, the sun compass, and path integration to navigate between their hive and food sources with remarkable precision. This allows them to optimize foraging efficiency and communicate resource locations to nestmates through the waggle dance. Similarly, desert ants (*Cataglyphis* spp.) traverse featureless landscapes using vector-based navigation, integrating distance and direction cues to return to their nest without relying on pheromone trails, a crucial adaptation to arid environments.

In vertebrates, spatial memory plays a central role in resource management and territorial behaviour (Broglio et al., 2003; Bingman, 2014; Geva-Sagiv et al., 2015; Hok et al., 2016). Rats use hippocampus-dependent spatial learning to navigate mazes and locate rewards, mirroring their natural ability to memorize complex burrow systems and food locations (O'Keefe and Dostrovsky, 1971; Moser et al., 2008). Food-caching birds like the Clark's nutcracker (*Nucifraga columbiana*) employ highly precise spatial memory to recall thousands of stored seeds, ensuring survival during winter. While mammals and birds have been extensively studied, ectothermic vertebrates such as amphibians and reptiles remain underexplored. However, evidence suggests that some reptiles use landmark-based navigation to relocate refuges, and amphibians can remember breeding sites over long distances, indicating that spatial cognition in these groups may be more sophisticated than previously assumed (Rodda & Phillips, 1992; Cayuela et al., 2014).

Reptiles in particular are an important outgroup in the vertebrate linage and exploring their cognitive abilities, alongside mammals and birds, offers valuable insights into the evolution of cognition. These three groups share a common amniotic ancestor, which raises the possibility that some behavioural traits or cognitive capabilities could have roots in their shared ancestry (Wilkinson & Huber, 2012). Researchers have begun to investigate the cognitive abilities of reptiles, shedding light on potential similarities and differences across species (De Meester & Baeckens, 2021; Szabo et al., 2021; Font et al., 2023). Particularly lizards provide a valuable opportunity to investigate how cognitive processes evolve in response to specific ecological demands (Wilkinson & Huber, 2012; Whiting & Noble, 2018). These animals utilize a variety of sensory modalities, including visual, olfactory, and tactile cues, to encode spatial information and solve complex directional tasks (Muheim et al., 2014; Szabo et al., 2021). Lizards exhibit flexible spatial sensory strategies depending on their ecological context. For example, some species integrate multiple types of cues to navigate effectively, such as landmarks, scent trails, and spatial environmental features (Vicente & Halloy, 2017; Kabir et al., 2020). In contrast, other species, particularly those inhabiting relatively homogenous environments like open grasslands, rely primarily on visual landmarks for orientation (Fleishman, 2024). Importantly, similar to mammals and birds, side-blotched lizards (*Uta stansburiana*) demonstrate spatial memory when navigating a maze and form a cognitive map to reach their goal (LaDage et al., 2012). Similarly, research on *Podarcis liolepis* confirmed the species' ability to locate a specific refuge among multiple options (Font, 2019). Differences in spatial cognition appear to be affected by habitat structure and ecological requirements, reflecting an adaptive evolution of cognitive strategies to overcome specific environmental challenges (Storks et al., 2023). Despite the growing research interest into lizard spatial learning and memory, there are still only a few studies that have addressed this topic (Reiter et al., 2017; Szabo et al., 2021). The mechanisms underlying spatial navigation in reptiles remain poorly understood and it is still unclear whether they can employ the same types of spatial learning processes observed in other vertebrate groups (Matsubara et al., 2017).

Cerebral lateralization, the specialization of functions in the left and right hemisphere of the brain, could play a significant role in spatial orientation and learning in animals. Research suggests that lateralization may enhance cognitive efficiency by allowing for specialized processing of spatial information, such as navigation and memory (Vogel et al., 2003). However, almost all studies that have investigated possible relationships between lateralization and spatial learning ability concern the human species and few other vertebrates (Levy, 1976; Ernest, 1998; Kessels et al., 2002; Prior, 2006; Oleksiak et al., 2011). In these cases, the right hemisphere is often associated with spatial processing, including the formation of cognitive maps, while the left hemisphere may be more involved in verbal or symbolic processing. The interplay between these hemispheres could influence how animals navigate their environment, process spatial cues, and learn new routes or landmarks.

In this study, we used an experimental arena-based approach in the form of a maze without immediate appetitive reinforcement (such as food or water) to investigate short-term spatial memory in common wall lizards (*Podarcis muralis*) and its relationship with lateralization, assessed through turning bias. In both the training and test phases, a shelter was present in the maze; however, during training it was simply part of the environment and lizards explored freely without predator cues. In the test phase, a standardized predatory stimulus was introduced, making the shelter an ecologically relevant goal linked to safety rather than to a food reward. *P. muralis* has been previously studied for its cognitive abilities, particularly in relation to lateralization in anti-predatory contexts. There is evidence that the right hemisphere is preferentially involved in escape responses and visual processing during predator avoidance (Bonati et al., 2010; Csermely et al., 2010). Given that lateralization is linked to asymmetric neural processing and decision-making, we hypothesise that individuals with a stronger turning bias, indicative of a higher degree of lateralization, exhibited differences in their ability to learn and navigate the maze.

Methods

Lizard collection and housing

Forty adult male lizards were captured by noosing (Bertram & Cogger, 1971) between March and May 2023 in San Genesio ed Uniti (province of Pavia, Lombardy region; 45°14′14.83″N, 9°11′13.04″E). The area is characterized by a mix of green spaces and terraced houses, contributing to a largely rural landscape. Lizards were measured (snout-vent length and tail length) using a dial calliper (±0.1 mm; range: 60-72 mm, mean ± SD: 66.6 ± 2.79) and weighted by a Pesola spring scale (±0.1 g; range: 5-8.5, mean ± SD: 6.66 ± 0.84). Tail condition was also assessed by recording if tail was complete, regrown or missing. We selected males since they are generally less negatively affected by captivity than females, especially during the breeding season (Galeotti et al., 2010). After capture and measurements, the lizards were transported inside individual bags to the laboratory, where each was assigned to an individual enclosure (Ferplast Faunabox, 35.6 × 23.4 × 22.8 cm), pre-arranged and placed in cabinets equipped with heating lamps (operating for 4 hours daily, from 9 AM to 1 PM) and UVB NEON lights (on for 8 hours daily, from 9 AM to 5 PM). Enclosures were furnished with absorbent paper on the bottom, a small water dish, a food dish, a terracotta tile, and mesh walls, designed to mimic a natural environment while facilitating cleaning and animal retrieval during the experimental phases. Every two days, the lizards were fed three mealworms (larvae of the flour beetle, *Tenebrio molitor*) each. The animals were housed in the laboratory for four weeks before the experimental tests began and were released back at the capture site once the research was completed.

Experimental design

The spatial learning and memory test was carried out in three temporal batches between the 4th of April and the 2nd of June 2023 (batch 1: N = 5, 4/4/23 to 6/4/23; batch 2: N = 4, 11/4/23 to 13/4/23; batch 3, N = 11, 30/5/23 to 1/6/23). Test trials immediately followed the last training trial on the next day (7/5/23 batch 1, 14/4/23 batch 2 and 2/6/23 batch 3; batches of control lizards were tested on the same days). The laterality test was carried out after the spatial learning and memory test between the 30th of April and the 9th of June 2023 (two batches, batch 1: N = 9, 30/4/23 to 4/5/23; batch 2: N = 11, 4/6/24 to 9/6/23).

Spatial learning and memory

Prior to the experiment, the 40 male lizards were randomly assigned to two groups: 1) an experimental group, consisting of 20 individuals, which were given repeated access to the maze prior to the test day to facilitate memorization of a shelter location; and 2) a control group, consisting of the remaining 20 animals, which were only exposed to the maze on the test day. The experiment was divided into two phases: maze exploration (days 1, 2, and 3 for the experimental group) and testing (day 4 for all animals). The experiments were scheduled to begin at 9:00 AM, with the heating lamps in the cabinets housing the terrarium set to turn on at 8:00 AM, ensuring the animals had at least one hour of light and warmth to become sufficiently active.

A novel maze structure (“Stag maze”; Fig. 1, C, D), specifically designed for this study, was developed with consideration of the ethology and ecology of lizards (Wenk, 1998; Sharma et al., 2010; Foreman & Ermakova, 2013). The maze was constructed with a polystyrene base covered in white plastic to create a smooth and even surface, measuring approximately 60 × 100 cm. Dark green plastic cut into rectangles of various sizes (seven measuring 10 × 25 cm, twenty measuring 25 × 25 cm, and two measuring 37 × 25 cm), were assembled and glued onto the base to form the maze structure. A gate (10 × 25 cm), made from a plastic rectangle, was added to delineate the starting area where the lizard would wait at the start of a trial. The gate was raised remotely using a drawbridge mechanism, operated by a nearby tripod. Two removable transparent plexiglass panels were placed over the entire maze to prevent the lizards from leaving the maze during trials. These panels were removed during maze cleaning and reinserted before each trial. To ensure uniform lighting and eliminate shadows within the arena, LED lights were installed along the entire upper perimeter of the maze (totalling 6.44 m). As a result, the room where the test was conducted remained dark, with the only illumination coming from the LEDs. Finally, a hiding spot, made from a terracotta tile, was placed as a shelter alternately in one of the last two branches of the maze, corresponding to arms 3 or 4 (in Fig. 1, C the shelter appears on the right, in arm 4). The shelter was available in the maze during both the training and testing phases. During training, however, it functioned simply as part of the maze structure and carried no immediate motivational value. In the test, the introduction of the predator stimulus (see below) gave the shelter a clear role as a safety refuge, rather than as a source of food. Throughout the trials, a camera was positioned above the maze to provide a full view of the arena and record the animals' performances. Each lizard was randomly assigned a fixed position for the shelter (either arm 3 or arm 4, Fig. 1, C), which remained consistent for the duration of the experiment for the experimental group, both during the exploration phase and the final test. The order in which animals within a day were tested was also randomized.

During the training trials (days 1–3), each experimental lizard was placed in the waiting area for 60 seconds without disturbance. After this period, the gate was raised remotely via a tripod to minimize the experimenter's proximity to the maze, thereby reducing potential stress and ensuring natural exploratory behaviour. The animal then had 20 minutes to explore the maze freely, before being removed and returned to its enclosure. During this time, the movements of the lizard were monitored remotely via a smartphone app, allowing for real-time tracking of which arms of the maze were visited. These data were recorded to confirm that, by the end of the three days, all animals had explored all arms of the maze and successfully found and entered the hiding spot. Before starting the next trial with another individual, the hiding spot was repositioned, and the plexiglass panels and gate were restored. On days 2 and 3, the same procedure as day 1 was followed, with the animals tested in a new random order.

Day 4 was designated for the actual test, which involved all 40 lizards, including those in the control group, which had not previously explored the maze. The animals were placed in the waiting area for 30 seconds without disturbance. Immediately after the gate was opened, a predatory stimulus was introduced. This stimulus consisted of the experimenter tapping his fingernails on the walls of the waiting room with a rotating hand movement. The stimulus continued until the lizard reached the first decision point in the maze, located immediately after the starting corridor. The trial was terminated when the animal either reached the shelter or after a maximum of 5 minutes. The role of the experimenter was critical to ensuring a consistent predatory stimulus. To minimize bias, the experimenter was blind to whether the animal belonged to the experimental or control group, preventing any inadvertent influence on the lizard's behaviour. After each trial, the maze was cleaned with absorbent paper and a 50%

alcohol solution to eliminate any scent traces that could influence the behaviour of subsequent animals.

For each lizard, during training trials (days 1–3), we recorded: 1) the latency to reach the shelter, measured from the opening of the door until the whole body was inside the shelter, and 2) the choice made at the first fork (right/wrong with respect to the shelter position). For the final test (day 4) we recorded: 1) the choice made at the first fork, 2) whether the lizard reached the artificial shelter or not (binary variable), and 3) the latency to reach the shelter, measured from the door opening. Lizards that did not reach the shelter within 20 minutes during training or 5 minutes during the test were given a censored latency of 1200 seconds and 300 seconds, respectively.

Lateralization

In addition to the spatial learning in the maze, all 20 animals from the experimental group were also tested for their behavioural lateralisation in a T-maze. The T-maze was made of green plastic, measuring 62 cm in length and 50 cm in width (Fig. 1, C, D). The maze featured a 12 cm wide corridor, where the lizard to be tested was placed at the start. The corridor was equipped with a sliding door, which could be raised remotely via a string. This setup ensured that the experimenters remained out of the animal's line of sight and that the door was consistently lifted in the same manner for all trials (Fig. 1, C). Similar to the spatial learning and memory test, lizards had 1h to warm up before testing.

Each lizard underwent three trials per day over the course of 5 consecutive days in a random order each day, resulting in a total of 15 trials per lizard, or 300 trials in total (20 animals × 15 trials). For each trial, the lizard was placed at the starting point of the maze, separated by a closed sliding door. After a 30-second waiting period, the door was raised, and simultaneously, the animal was exposed to a predatory stimulus. The experimenter simulated the threat by moving their hand above the lizard, and touching the maze walls to generate noise. The lizard typically fled away from the stimulus, heading toward the maze fork and turning either left or right. The direction of the turn was recorded as the primary variable of interest. Each test was filmed using a Canon Legria video camera. The experiment was conducted in a quiet, dimly lit room, with the maze illuminated uniformly by a light source above it. To standardize the threat simulation, the same experimenter simulated the predatory stimulus for all trials. Lizards were not habituated to the maze prior to testing. After each trial, the maze was carefully cleaned with 50% ethanol to eliminate any residual scent traces that might influence the behaviour of the next animal.

Data analysis

We initially explored the potential effects of tail length and condition (complete, regrown and missing) on lizard lateralization and learning performances in the experimental group. Since lizards' tail grew during the permanence in our lab, we estimated the tail length of each lizard at the time of testing. This was necessary because variations in tail morphology can influence locomotion and balance and may, in turn, affect performance, potentially acting as a confounding factor in behavioural tests. We first calculated the individual daily tail growth rate using the difference in tail length at capture and release, divided by the number of days between these two timepoints. This provided a per-day tail growth rate for each individual. We then estimated the tail length at the time of the test by multiplying the growth rate by the number of days between capture and test and adding this to the initial tail length at capture. This approach assumes a linear growth trajectory of tail regeneration over time and allows for an estimation of tail length for use in subsequent models. Estimated tail length and condition were then used as predictors in Bayesian generalized linear mixed model (BGLMM, package *brms*; Bürkner 2017, 2018, 2021) to assess their possible influence on latency to reach the shelter or in lateralization. We also investigated the potential effect of the position of the shelter during learning phase and test.

Lateralization

To assess the degree of lateralization in each individual and the preference for a specific escape direction, we employed a BGLMM with Bernoulli distribution and logit link function. The binary side choice (right = 1, left = 0) for each trial was used as the response variable; the model did not include fixed effects (null model), while the individual lizard was included as the random effect, accounting for repeated measurements within individual.

Spatial Memory, Learning and Laterality

To evaluate if lizards that experienced training for three days had learnt to navigate the maze, we compared both the probability to reach the shelter and time taken to reach the shelter between experimental and control animals. To compare the probability to reach the shelter, we used a Bayesian generalized linear model (BGLM) with Bernoulli distribution. The response variable was a dichotomous outcome, where a value of 1 indicated successful arrival at the refuge within 300 seconds of the test, and a value of 0 represented failure to reach the refuge. The fixed effects included the treatment group, a two-level factor distinguishing between the control and experimental groups. No random effect was used as animals only received one test trial.

Similarly, we compared the time taken to reach the shelter between the experimental and test group (fixed effect) using a censored BGLM (Bayesian generalized linear model) with log-normal distribution. The only fixed effect was treatment group and no random effect was included. Finally, we evaluated learning by investigating the change in time taken to reach the shelter across the three training trials and the test trial using a BGLMM. This model also evaluated the change in time taken to reach the shelter across trials, the relationship to the strength of laterality and their interactions (fixed effects). We calculated the relative lateralization index as $\frac{R-L}{R+L} \times 100$ (RLI), where R and L are the number of choices toward the right and left directions, respectively, which reveals the sample's directional preference, and the absolute lateralization index (ALI) as $\left| \frac{R-L}{R+L} \times 100 \right|$, which measures the strength of lateralization, or the degree of asymmetry without considering the direction. We included the ALI rather than the RLI because few animals showed lateralised behaviour to either the left or right side in the laterality test. We included a random effect of animal identity to account for repeated testing of the experimental lizards during training. Additionally, we explored the relationship between the strength of lateralization and the time taken to reach the shelter. For the learning trials (days 1–3), we fitted a BGLMM with trial number and lateralization strength as fixed effects and the individual as a random intercept. For the final test (day 4), where each lizard was tested only once, we used a BGLM with the same fixed effects but no random term.

To assess the probability of making the correct choice at the first crossroad during the learning phase (Trials 1–3), we fitted Bernoulli logistic regression models including trial number as a fixed effect and individual identity as a random effect to account for repeated measures. For the final test trial, where each lizard contributed only one observation, we used a Bernoulli model without random effects to estimate the mean probability of making the correct first choice.

All statistical analyses were run in R version 4.4.2 (R Core Team, 2024). We used a generic weakly informative normal prior with a mean of 0 and a standard deviation of 1 and ran 4 chains per model of 5000 iterations each and a thinning interval of 1 (default settings). We made sure that model Rhat was 1, that the ESS was above 2000 and checked the density plots and correlation plots to ensure that the models had sampled appropriately. We provide Bayes factors (BF) to evaluate the results by determining Bayes Factors from marginal likelihoods using the package *brms*. Bayes factors below 1 indicate no difference while above 1, BF indicate support for a difference (Schmalz et al., 2023).

Ethics and permits

The methods applied were non-invasive and followed the guidelines provided by the Association for the Study of Animal Behaviour/ Animal Behaviour Society for the treatment of animals in behavioural research and Teaching (ASAB Ethical Committee and ABS Animal Care Committee, 2023). The method used to capture the lizards (noosing) is commonly employed due to its effectiveness and because it does not cause harm to the animals (Fitzgerald, 2012). In our study, all animals were kept in indoor conditions that closely resembled their natural environment. During the trials, the lizards showed no signs of stress, and at the end of the experiments, they were returned to their original capture sites. All animals maintained their initial body mass throughout the study and were released back at their initial capture site after the experiment had concluded. Animal collection, husbandry, and testing were approved by the Ministry of Ecological Transition (ISPRA Prot. 0010736/2022, 01/03/2022).

Results

Lateralization

Ten individuals preferred the right direction more frequently during the 15 trials conducted, while the remaining 10 chose the left direction more often (Fig. 2). However, model estimates suggested a preference in only 7 individuals (35%), of which 3 preferred the right direction and 4 the left direction (Fig. 2). Overall, the group average did not reveal significant group-level bias in the direction of lateralization during escape (BGLMM, estimate = -0.006, CI_{low} = -0.646, CI_{up} = 0.673; Fig. 2).

Spatial Memory, Learning and Laterality

We found strong evidence for a higher probability to reach the shelter during the test trial in lizards from the experimental group compared to the control group (BGLM, estimate = 1.721, CI_{low} = 0.551, CI_{up} = 2.948, BF = 36.6; Fig. 3). We also found very strong support for a lower latency to reach the shelter during the test trial in lizards from the experimental group with respect to the control group (BGLM, estimate = -1.632, CI_{low} = -2.530, CI_{up} = -0.721, BF = 131.78; Fig. 3). We found no support for an effect of an interaction between trial and the strength of lateralization (BGLMM, estimate = 0.360, CI_{low} = -0.483, CI_{up} = 1.176, BF = 0.59) on the latency to reach the shelter during training; the interaction was subsequently removed from the model. After removing the interaction, we found strong evidence for a decrease in the latency to reach the shelter across trials (BGLMM, estimate = -0.488, CI_{low} = -0.815, CI_{up} = -

0.149) and for a positive relationship between the latency and the strength of lateralization (BGLMM, estimate = 1.493, CI$_{low}$ = 0.348, CI$_{up}$ = 2.586, BF = 256), showing that more strongly lateralized individuals took longer to reach the shelter during the initial learning trials. In particular, the slope of the lateralization index was positive and statistically credible in both Trial 1 (β = 1.29, CI$_{low}$ = 0.13, CI$_{up}$ = 2.41) and Trial 2 (β = 1.59, CI$_{low}$ = 0.01, CI$_{up}$ = 3.18), indicating that individuals with stronger lateralization took longer to reach the shelter. However, this effect was no longer supported in Trial 3 (β = 1.24, CI$_{low}$ = –0.33, CI$_{up}$ = 2.78) and was effectively absent in the final test trial (Trial 4: β = 0.07, CI$_{low}$ = –1.54, CI$_{up}$ = 1.64), which was confirmed in our analysis of the test trial only, in which we found no clear relationship between lateralization and latency to reach the shelter (estimate = -0.138, CI$_{low}$ = -1.677, CI$_{up}$ = 1.370, BF = 0.79).

Furthermore, only weak support emerged for an effect of trial number on the probability of making the correct choice at the first crossroad during the learning phase, with the model including trial as a predictor showing only a slight advantage over the null (BF = 1.60). In the final test, lizards in the experimental group were estimated to choose the correct path on their first turn about 71% of the time (0.919, CI$_{low}$ = 0.093, CI$_{up}$ = 1.819).

Effects of tail length and condition

Both tail length and condition did not affect the latency to reach the shelter for the experimental group during the test (BF = 0.177 and 0.458 respectively). The shelter position did not affect the latency to reach the shelter (BF = 0.670). During the learning phase, tail length and condition showed no detectable effects on latency (BF = 0.241 and 0.043 respectively); moreover, the position of the shelter (side) did not influence the latency to reach it during the learning phase (BF = 1.00). Neither tail length nor tail condition had a detectable effect on lateralization in the experimental group. The comparison between models and their respective null models indicated stronger support for the latter in all cases. For absolute lateralization, the Bayes factors were 0.092 for tail length and 0.283 for tail condition, suggesting substantial to moderate evidence against any effect. Similar patterns emerged for the raw lateralization index (LI), with Bayes factors of 0.189 for tail length and 0.617 for tail condition.

Discussion

We found that experimental lizards were able to learn to navigate a complex maze within three days indicated by a reduction in the time taken to reach the goal shelter and increase in the probability to reach the shelter compared to control lizards without experience of the maze. We also found that some lizards showed lateralised behaviour in a T-maze but as a population they did not show lateralised behaviour to a specific side. Finally, we found that more strongly lateralised lizards took longer to reach the goal shelter during training.

The main aim of this study was to investigate the relationship between lateralised turning behaviour and the navigation of a complex maze in a lizard known to rely on different hemispheres for processing different types of information (Bonati et al., 2010; Csermely et al., 2010). We found a relationship between turning behaviour and maze learning; lizards that were more strongly lateralized (to either side) took longer to reach the goal shelter during training. This result indicates that lateralised turning behaviour might interfere with arm choices made during exploration increasing the time until the shelter is found. However, in the test trial, no clear relationship was found between lateralised turning behaviour and the latency to reach the shelter. This suggests that lizards may learn spatial information similarly regardless of their level of lateralization, and that any potential advantage of lateralised turning behaviour could only emerge when the context of a threatening situation is matched across experiments, but our data do not provide strong evidence of this. During training, lizards were not threatened but left free to explore the maze. While during the test trial, as in the lateralisation experiment, they were presented with a threatening stimulus (a human hand). This contextual similarity may have activated comparable brain areas, leading to the observed improvement in performance. Importantly, a range of studies show that strongly lateralised individuals outperform less lateralised individuals only under certain conditions. For example, in chicks (*Gallus gallus domesticus*), strongly lateralised individuals remain proficient in a foraging task under predation pressure, while weakly lateralised individuals' performance in the foraging task decreases under pressure (Rogers et al., 2004). Similarly, more strongly lateralised *Girardinus falcatus* fish capture prey faster under predation pressure than weakly lateralised fish (Dadda and Bisazza, 2006a), and more strongly lateralised females *G. falcatus* retrieved a reward faster when harassed by a male (Dadda and Bisazza, 2006b). In conditions without pressure, both groups (strongly and weakly lateralised individual fish and females) perform the same (Dadda and Bisazza, 2006a; 2006b). All these examples involve a foraging task, while our test involved spatial learning of a route to a shelter. Further studies are, therefore, needed to better understand how pressure plays a role in maze learning in relation to behavioural lateralisation.

The comparison of behaviour between the experimental and control group revealed that lizards receiving three trials of experience with the maze were more likely and faster in reaching the shelter. Additionally, over trials, individuals from the test group became faster at reaching the shelter. Together, this demonstrates a fast acquisition of spatial information in these lizards. In only three days, lizards acquired enough information to learn the location of the shelter. Reptiles such as lizards, are often regarded as slow and sluggish and mostly driven by innate behaviour and less so by cognitive processes (Burghardt, 2013; Font et al., 2023). Over the last few decades, however, research has accumulated showing that this is not the case (Szabo et al., 2021). As an outgroup in the vertebrate lineage, research on the cognitive abilities in lizards can shed new light on the evolution of cognition and due to their large diversity (Pianka and Vitt, 2003), lizards are excellent models for comparative cognition research. Our results support the use of lizards and demonstrate fast and proficient spatial learning in *P. muralis*.

Despite lizards showing behavioural lateralisation, we found that only seven of the 20 tested individuals were lateralised to one side and found no discernible trend across the whole sample. Similar results have been obtained across a wide range of taxa (Vallortigara and Bisazza, 2002). We would like to point out that our lizards originated from the wild, therefore, this pattern might demonstrate natural variation in turning behaviour if our subsample is representative of the population of origin. Of the seven individuals that showed a clear lateralisation, four were lateralised to the left and three to the right. Due to this low sample, we are unable to determine if right or left lateralisation was more beneficial. Processing spatial relations has been shown under the control of the right hemisphere with humans and chimpanzees having larger hippocampal volume in the right hemisphere (Vallortigara and Versace, 2017). Future studies using a larger sample could compare maze learning between left and right lateralised individuals.

Across studies, evidence indicates that stimuli in threatening contexts (e.g., escaping predation) are processed primarily through the left eye/ right hemisphere, whereas food-related stimuli are processed through the right eye/ left hemisphere (Vallortigara and Versace, 2017). Results from previous studies in *P. muralis* follow along those lines. Bonati and colleagues (2010) showed that out of 21 wild caught male and female *P. muralis*, only six showed a preference for escaping to one side after a simulated predator attack; five to the right (using the left eye to monitor predators) and one to the left. In a second experiment using a modified Y-maze, only two lizards showed lateralised behaviour choosing the right arm more frequently than the left. When detouring a barrier to reach prey, only seven out of 16 wild caught males individuals were lateralised, six turning preferentially to the left (monitoring the prey with the right eye) when detouring the barrier and only one to the right (Csermely et al., 2010). Our own results are not so clear. Even though animals were tested in a predatory context and we would expect them to turn more frequently to the right, we found about equal numbers of animals showing a left/ right turning bias. Furthermore, when exploring a maze, wild caught *P. muralis* males and females previously showed a 100% left side bias on their first turn when unfamiliar with the maze and no threat present (Csermely et al., 2011). In contrast, our data show that in the first trial of training, 50% of our individuals turned to the left and 50% to the right. These differences may reflect variations in housing or testing methodology but could also be related to population of origin. Future studies could compare individuals from different populations under the same testing methodology to assess how lateralised behaviour varies across populations.

In summary, we found that more strongly lateralised lizards took longer to reach a goal shelter when exploring an unfamiliar maze but showed no clear disadvantage when escaping a threat within the same maze after learning the route. To the best of our knowledge, this is the first time that lateralised turning behaviour was linked to maze learning in a reptile species. Remarkably, just three training trials were sufficient for lizards to acquire the information needed to escape a simulated predator attack into a goal shelter, demonstrating that they can learn to navigate a maze with minimal training. Our findings open new avenues for research on the role of brain lateralisation in spatial learning across reptiles inhabiting different ecological contexts.

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Data availability statement: Data generated during this study are available for download from the Open Science Framework (OSF, link for review purposes: https://osf.io/af94r/?view_only=6796abf0197e44c3b809b2902b076618).

Code availability statement: All code generated to analyse the collected data is available for download from the Open Science Framework (OSF, link for review purposes: https://osf.io/af94r/?view_only=6796abf0197e44c3b809b2902b076618).

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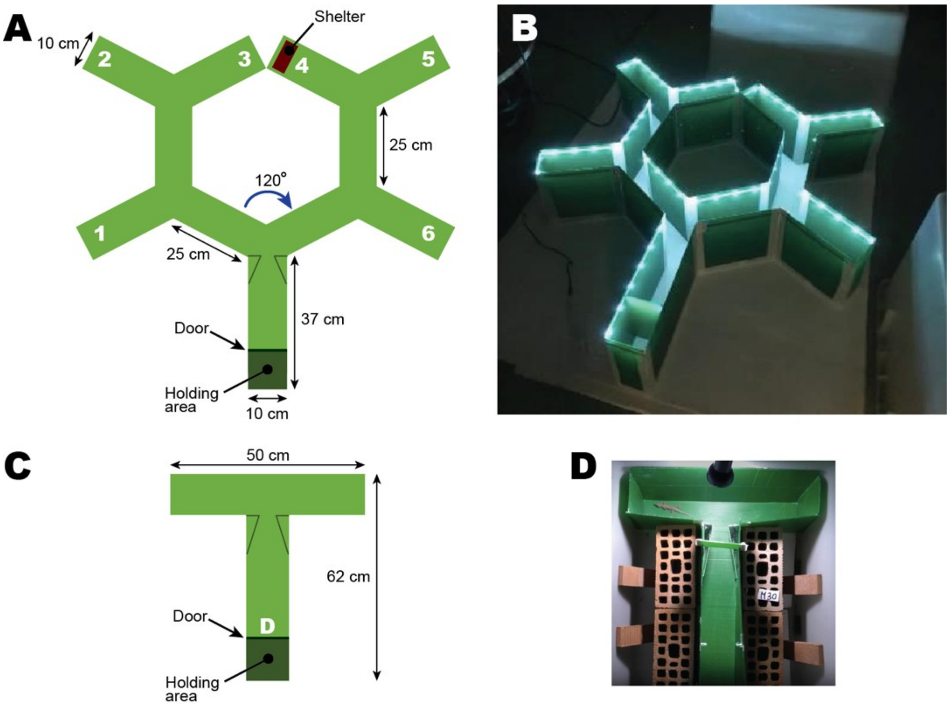

Figure 1. Schematics and pictures of the mazes used for the spatial learning and memory (A, B) and laterality test (C, D). (A) Schematic of the "Stag maze" used to test spatial memory. The maze included 6 arms of which one provided a shelter (either in arm 3 or 4) as the goal for lizards in the experimental group. Animal were held in a holding area at the start of the experiment and could enter the maze after a door was lifted. (B) Picture of the "Stag maze" with the door closed. LEDs covered the entire perimeter of the structure to achieve even illumination of the whole maze. (C) Schematic of the T-maze used to test behavioural lateralisation in male *P. muralis*. The animal were held in a holding area at the start of the experiment and could enter the maze after a door was lifted. (D) Picture of the T-maze with a male *P. muralis* in the left arm. The maze was illuminated from the top with a single light bulb.

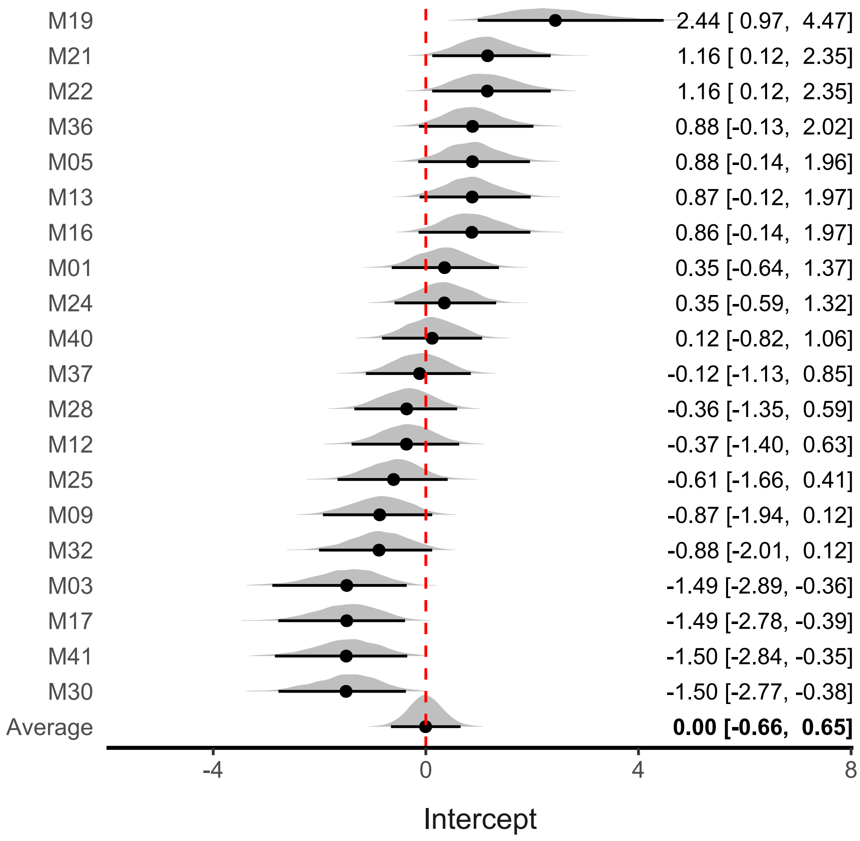

Figure 2. Results of the Bayesian analysis of logistic regression on lizard escape direction choice in the laterality test (experimental group: N = 20). Each point represents the estimated random effect for individual lizards (log-odds), and horizontal black bold lines indicate the 95% credibility intervals. The vertical red, dashed line represents neutrality (log-odds = 0), where there is no preference for a specific escape direction (right or left). Density curves in the background (grey) represent the distribution of escape direction choices recorded for each individual across 15 tests. Points whose credibility intervals do not intersect the dashed line indicate individuals with a significant preference for either the right or left direction.

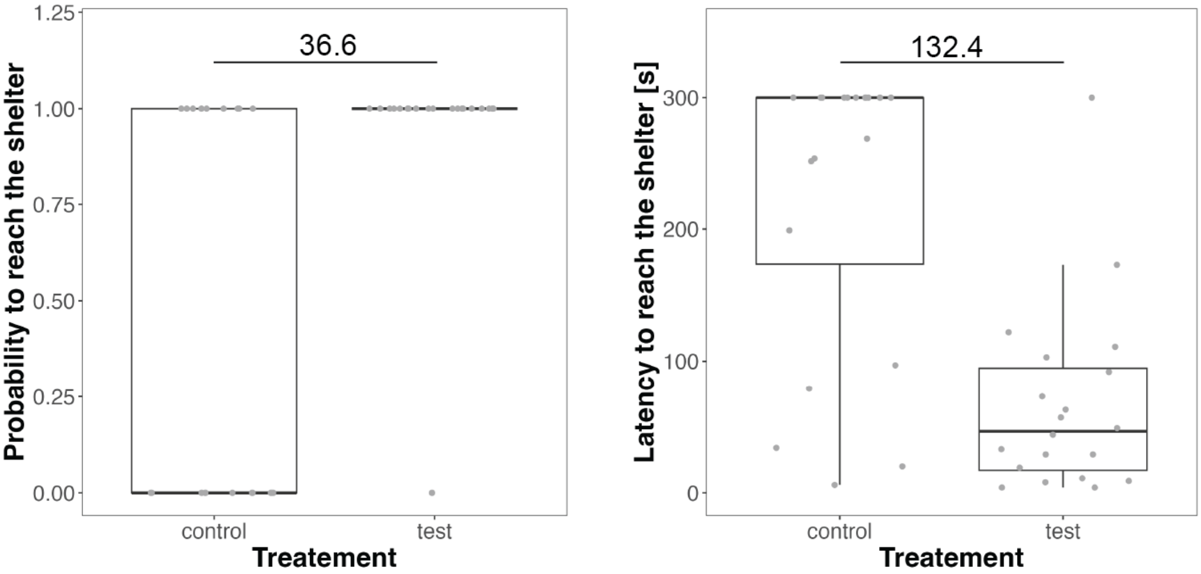

Figure 3. Boxplot of the probability to reach the shelter (left) and the time taken to reach the shelter (right) of control lizards (did not receive training in the maze) and experimental lizards (received 3 trials training in the maze). The bold line within the boxes represents the median, the upper and lower box edges show the upper and lower quartile, respectively. The ends of the whiskers show the maximum and minimum. Grey dots indicate individual lizards' performance (control: N = 20; experimental: N = 20). Lizards that did not reach the shelter within 5 minutes (300 seconds) were given a censored latency of 300 seconds. Numbers above lines indicate Bayes factors.

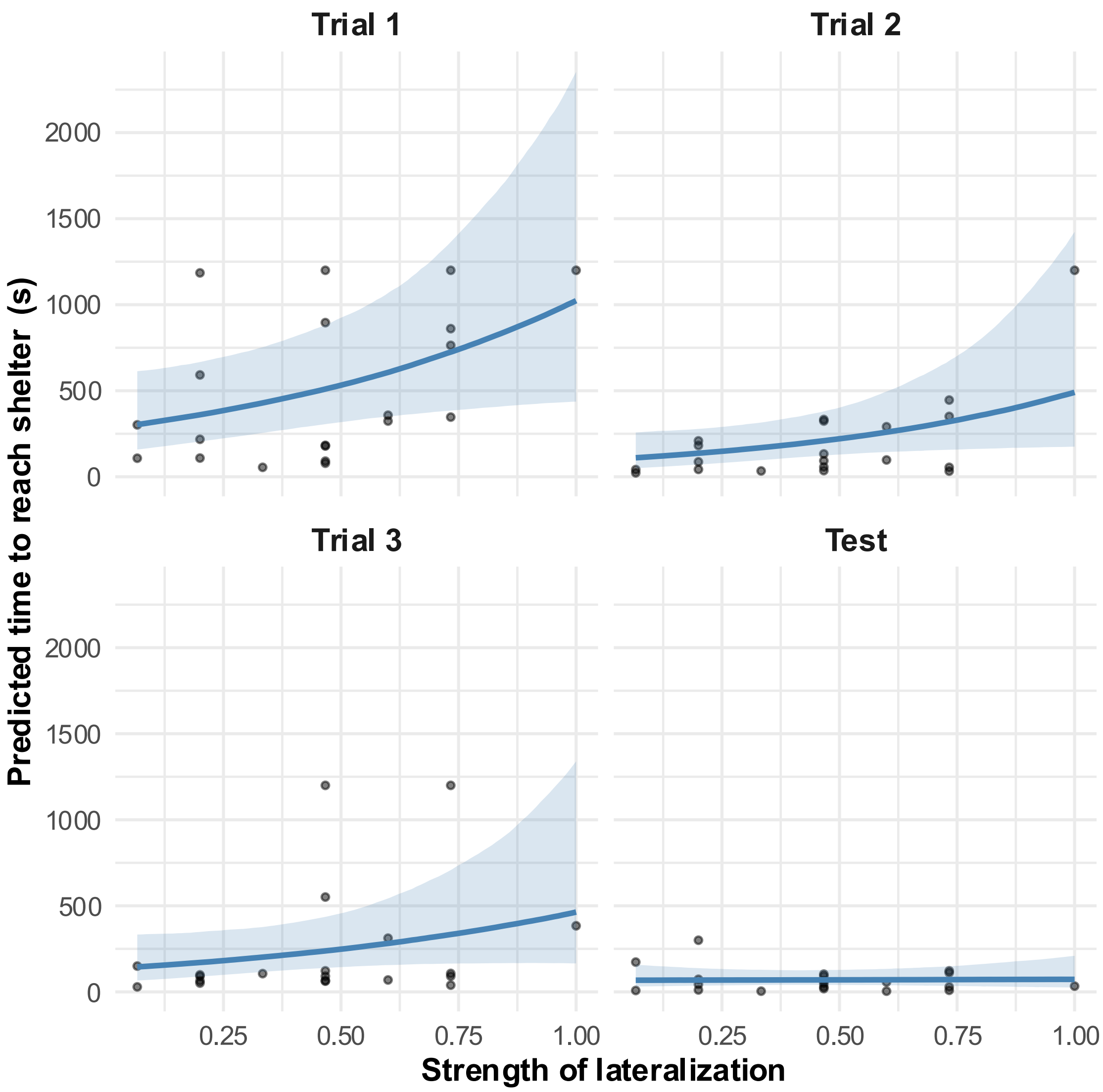

Figure 4. Predicted relationship between lateralization strength and the latency to reach the shelter across the four experimental trials (n = 20). Each panel displays a separate trial (training trials and the test). Grey points represent individual observations. The blue lines show predicted values from the Bayesian regression model, and the shaded areas correspond to 95% credible intervals. A positive association between lateralization strength and shelter latency is evident in the early trials, but this effect weakens in Trial 3 and is no longer present in the test phase.