Behavioural Brain Research 518 (2027) 116556

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research article

Complementary hemispheric functions during spontaneous exploration in Hermann's tortoises

Orsola Rosa-Salva [a,b,*], Emanuela Musto [a], Anastasia Morandi-Raikova [a,b], Maria Elena Miletto-Petrazzini [c], Gionata Stancher [a,b,d], Angelo Bisazza [e], Valeria Anna Sovrano [a,b,**]

[a] CIMeC Center for Mind/Brain Sciences, University of Trento, Italy
[b] Department of Psychology and Cognitive Science (DIPSCO), University of Trento, Italy
[c] Department of Biomedical Sciences, University of Padova, Italy
[d] Rovereto Civic Museum Foundation, Italy
[e] Department of General Psychology, University of Padova, Italy

ARTICLE INFO

Keywords:
Lateralization
Reptiles
Tortoises
Spontaneous exploration
Hemispheric specialization
Testudo hermanni

ABSTRACT

Brain lateralization is a widespread feature of vertebrate nervous systems, yet evidence from reptiles remains limited. We investigated behavioural lateralization in Hermann's tortoises (*Testudo hermanni*) during spontaneous exploration of a novel environment. Forty-six adult tortoises were observed in a rectangular arena allowing free exploration of two lateral end-zones connected by a central corridor, over three sessions, revealing a dissociation in the direction of lateralization across different behavioural measures. Tortoises showed a rightward bias in gaze direction. Automated tracking of animals' position also revealed that males alternated more often between the neutral zone and the right side, suggesting a more cautious or monitoring-type exploration in that direction. In contrast, measures associated with active exploration showed a leftward tendency: animals spent longer touching the left corner and, in males, entered the left end-zone more frequently. The dissociation between rightward attentional biases and leftward exploratory behaviour is consistent with models proposing complementary hemispheric functions, in which the left hemisphere contributes to sustained visuomotor control whereas the right hemisphere supports rapid responses to salient stimuli. Overall, these findings provide new evidence for functional lateralization in chelonians and contribute to a comparative understanding of hemispheric specialization across vertebrates.

1. Introduction

Once considered unique to humans, brain lateralization is now known to be a widespread feature among non-human animals, including both vertebrates and invertebrates [1–9]. The presence of distinct functional specializations in the left and right brain hemisphere thus seems to be a fundamental organizational principle of the central nervous system, conferring important advantages to lateralized brains. For example, the specialization or dominance of corresponding structures in the two hemispheres for different functions may enhance computational efficiency by avoiding the unnecessary duplication of neural tissue devoted to the same processes [10]. Moreover, in animals with laterally placed eyes, in which each eye sees a different portion of the visual scene, a dominant hemisphere for specific tasks reduces the risk of interference between conflicting responses initiated by the two sides of the brain [7,11–13]. Lateralization can also improve performance in dual-task situations, as each hemisphere can focus on one of two simultaneous demands [14,15].

Importantly, in many animal species, the direction of lateralization is aligned at the population level. That is, for several cognitive and behavioural functions, the majority of individuals show the same dominant hemisphere, rather than being randomly distributed between left- and right-biased patterns. This population-level asymmetry has been documented across a broad range of taxa and behavioural domains (for reviews see; [6,7,9,13,16]). One influential interpretation is that this alignment reflects the demands of social coordination: when

* Corresponding author at: CIMeC Center for Mind/Brain Sciences, University of Trento, Italy
** Corresponding author at: Department of Psychology and Cognitive Science (DIPSCO), University of Trento, Italy.
E-mail addresses: orsola.rosasalva@unitn.it (O. Rosa-Salva), valeriaanna.sovrano@unitn.it (V.A. Sovrano).

https://doi.org/10.1016/j.bbr.2026.116556
Received 11 June 2026; Received in revised form 12 October 2026; Accepted 29 October 2026
Available online 4 November 2026
0166-4328/© 2026 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

individuals interact, predictability in the direction of lateralized responses may facilitate synchrony, reduce conflict, and enhance the efficiency of group-level behaviours [17,18]. From this perspective, population-level lateralization can be understood as an evolutionarily stable solution that balances the neural advantages of hemispheric specialization with the ecological and social benefits of coordinated action [16,19].

Across vertebrate taxa, several studies have reported recurring alignments in the dominant functions of the left and right hemispheres. Much of this evidence derives from research on species with laterally placed eyes, in which hemispheric specialization can be inferred by selectively presenting stimuli to one eye or by analysing spontaneous eye-use preferences during stimulus inspection. In such paradigms, differential behavioural responses following left- or right-eye exposure, as well as consistent biases in monocular viewing, have been interpreted as reflecting functional asymmetries between the contralateral hemispheres. Another approach to investigating hemispheric specialization consists in analysing the preferential side from which an animal chooses to initiate or execute a given behavioural response. Although species-specific adaptations and task-dependent discrepancies are well documented, a number of common trends emerge. Broadly speaking, the right hemisphere is often associated with rapid detection of biologically relevant or novel stimuli (e.g., responses to predators and conspecifics) and with the initiation of context-appropriate responses. In contrast, the left hemisphere has been linked to sustained attention, fine visuomotor control, and the regulation of routine or well-established behaviours towards familiar stimuli (e.g., foraging behaviours). These functional asymmetries have been described in birds, fish, reptiles, and mammals (e.g., [20–31]), suggesting the presence of evolutionarily conserved organizational principles, even if their behavioural expression may vary depending on ecological niche and life history. In an attempt to provide a coherent interpretative framework for these converging findings, Andrew [32] proposed a global model of hemispheric specialization. Rather than focusing on specific tasks, this model emphasizes broader functional domains, distinguishing between a right-hemisphere system specialized for readiness to respond to releaser stimuli and novelty, and a left-hemisphere system specialized for the visual control and maintenance of ongoing responses. The left hemisphere has a superior ability to sustain an initiated response to visual stimuli. This allows a specialization for the control of routine feeding behaviours and analysis of visual information in familiar contexts, counteracting distraction by irrelevant response to novelty. On the other hand, the right hemisphere is specialized for the response to releasers of species-specific behaviours and to novel stimuli, enacting primitive weariness and avoidance reactions.

Studying lateralization in reptiles could be particularly informative on the evolutionary roots of cerebral lateralization in vertebrates, since they likely preserve important features of the common ancestor they shared with birds and mammals, possibly including aspects of neural architecture. Although studies in reptiles remain relatively limited, a pattern of functional lateralization broadly consistent with the global model outlined above has been reported in several species. Much of the earlier work has focused on lizards, examining asymmetries in predatory behaviour (e.g., [33,34]), escape responses (e.g., [21,22,35]) or exploratory behaviour (e.g., [36,37]). Of particular relevance to the present study, investigations of exploratory behaviour in neutral environments—namely in the absence of prey, predators, or social stimuli—have reported right-hemisphere dominance in lizards ([37], see also [36]). This pattern was expressed as a leftward turning bias during maze exploration and as a preference for using the left eye when inspecting the open portion of a terrarium [37]. Such findings have been interpreted as reflecting right-hemisphere specialization for vigilance and monitoring functions, consistent with the general framework outlined above [32]. More specifically, Csermely et al. [37] interpreted these findings as reflecting right-hemisphere specialization for supporting vigilance and environmental monitoring, including the processing of spatial information and the maintenance of a broad, global attentional focus on the immediate surroundings. Intriguingly, however, these effects were modulated by the ecology of the different lizard species and by the sex of the individual animals [36,37], suggesting an adaptive role of these lateralization biases in relation to species-specific ecological demands and life-history strategies.

Only a few studies have focused on chelonians—that is, turtles and tortoises belonging to the order Testudines. In 2006, a seminal study by Stancher and colleagues reported the first evidence of motoric lateralization in chelonians, by showing that two subspecies of Hermann's tortoises have a rightward bias for overturning from a belly-up position [38]. However, conflicting results were subsequently found in some aquatic turtle species, suggesting that this form of motor lateralization could reflect species-specific factors [39,40].

Recent studies have revealed other forms of behavioural lateralization in chelonians, which seem to reflect a similar pattern of hemispheric specialization as known in other vertebrates—and broadly consistent with the model proposed by scholars such as Richard Andrew [32]. For instance, Hermann's tortoises tend to use the left eye to inspect a mirror [41], in line with the widespread evidence of right-hemisphere dominance for encoding social information (e.g., see [42] for a review). In contrast, in the absence of the mirror they rely more on the right eye to inspect their surroundings, possibly reflecting the involvement of the left hemisphere in action regulation and in the classification of stimuli, as well as in the processing of information that requires evaluating alternative behavioural options. In this condition, the test arena constituted a novel, neutral, and relatively uniform environment, where the animal had to decide how to move from the starting position while simultaneously categorising this unfamiliar situation [41]. In the same study, however, tortoises showed a bias to use the right forelimb when initiating movement in the presence of mirrors, a finding that appears to contradict the functional dissociation outlined above. This pattern was instead interpreted as supporting the so-called valence hypothesis, according to which the left hemisphere is preferentially involved in processing stimuli with a positive emotional valence [43]. This interpretation was based on the observation that, even in a relatively solitary species such as Hermann's tortoise, conspecifics may represent a positive stimulus: during the test, the animals were consistently attracted to the mirrors, which reflected their own image [41].

Additional evidence supporting the general dissociation of functions between the two hemispheres, as described in models such as that proposed by Andrew [32], has also been reported in other chelonian species. For instance, Aldabra giant tortoises (*Aldabrachelys gigantea*) show a tendency to rest with the head oriented to the right side, leaving the left eye exposed to the environment, a posture interpreted as reflecting preferential engagement of the right hemisphere in vigilance and monitoring of potential social or environmental threats [44]. Similarly, European pond turtles (*Emys orbicularis*) exhibit a left-side bias in escape and righting responses in antipredator contexts, consistent with a dominant role of the right hemisphere in the control of defensive behaviours [45].

Taken together, these findings provide several lines of evidence broadly consistent with the functional dissociation between the two hemispheres proposed in models such as that of Andrew [32]. At the same time, however, the available literature also reports results that appear partly inconsistent with this framework, suggesting that hemispheric specialization may be modulated by ecological context, behavioural domain, or species-specific adaptations. In this context, the present study offered the opportunity to investigate lateralization in Hermann's tortoises during a task of spontaneous exploration in a novel environment. By analysing different behavioural components of exploration, we aimed to assess potential asymmetries at multiple levels, including attentional biases (gaze direction), motor biases (approach and tactile interaction with the environment), and spatial exploration strategies (entries into different areas and alternations between them).

The primary aim of the study was to examine the presence and

direction of behavioural lateralization during spontaneous exploration of a novel environment in *Testudo hermanni*, in relation to the model of hemispheric specialization outlined above (e.g., [32]). In addition, we pursued several secondary objectives. First, we tested whether the strength or direction of lateralization was modulated by the sex of the animals. Second, we assessed the stability of any observed asymmetries by comparing behaviour during the first exposure to the apparatus, when no salient stimuli were present, with behaviour across subsequent sessions. Third, we combined manually coded behavioural measures with automated tracking variables to obtain a comprehensive characterization of exploratory behaviour. Finally, by considering distinct behavioural components of exploration, the study also allowed us to explore the possibility of a functional dissociation between attentional biases (e.g., gaze direction) and motor or spatial exploration patterns (e.g., touching, entries, and alternations), which could be interpreted in light of the hemispheric framework outlined above.

2. Materials and methods

This experiment was part of a broader, as yet unpublished, study investigating amodal completion in Hermann's tortoise (*Testudo hermanni*), and also aimed to assess behavioural lateralization through the analysis of spontaneous responses and side biases.

2.1. Ethics statements

The present research was conducted at the SperimentArea natural estate (Rovereto Civic Museum Foundation, Trento, Italy) through a scientific collaboration between CIMeC (Center for Mind/Brain Sciences) at the University of Trento (Italy) and the Rovereto Civic Museum Foundation. Zoological and experimental procedures complied with European legislation for the protection of animals (Directive 2010/63/EU) and adhered to Italian and EU laws regarding protected wild species (Art. 8/bis 150/92 Annex A Reg. (EC) 338/97). The experimental protocol was approved by the Internal Ethics Committee of the Rovereto Civic Museum Foundation (Protocol No. 0000317–2022).

2.2. Experimental subjects

A total of 46 *naïve* adults (age range 30–50 years) of *Testudo hermanni* were observed (24 of which were males). Sexual dimorphism in size, shell shape, and other anatomical features were used to determine sex [46]. Mean carapace dimensions were 16.56 ± 0.78 cm × 14.09 ± 0.99 cm (length × width) in males and 17.46 ± 1.22 cm × 13.33 ± 0.87 cm in females. Width was measured at the widest point of the carapace.

The animals were housed at the SperimentArea facility (Rovereto, TN, Italy). In this protected area, tortoises live in semi-naturalistic conditions within dedicated pens, have access to wooden shelters and water *ad libitum*, and are regularly fed with vegetables and fruit. The study took place in two consecutive years (2022 and 2023), from May to October.

Before behavioural observations began, the experimental animals were marked on their carapace with a white correction pen to facilitate individual identification. The animals were then placed in smaller enclosures, with at least two individuals per enclosure. Males displaying aggressive behaviour were isolated to prevent injuries, though they could still see and smell their conspecifics through a metal mesh. All enclosures were approximately 25 cm high from ground level.

2.3. Apparatus

The experimental apparatus, located inside a wooden hut at the SperimentArea facility, consisted of a rectangular white wooden arena (163 cm long x 83 cm wide x 41.3 cm high) and was a modified version of the apparatus used by Baratti et al. [47] to investigate spatial abilities in *T. hermanni*. Inside the arena, a central corridor (80 cm long x 29 cm wide x 10 cm high) was built using white plastic material (Poliplak®). The corridor had a large opening at each extremity, creating two hexagonal areas (36 cm wide x 19 cm long) (Fig. 1). Since this experiment was part of a larger study aimed at investigating amodal completion, during the second and the third session of the experiment, two pairs of stimuli typically used to test this phenomenon were located on the walls of the apparatus at the two corners of each hexagonal area (see Supplementary materials for details). However, as the animals' behaviour was not influenced by the presence, position, and the nature of the stimuli, this factor was excluded from further analyses.

A layer of pellets covered the floor of the apparatus, to improve traction during movement. A 400-Watt halogen lamp, positioned 115 cm above the centre of the apparatus, provided illumination and heating. Two webcams (Microsoft LifeCam Studio), mounted at heights of 110 cm and 120 cm, recorded the tortoises' behaviour during each session.

2.4. Procedure

All the 46 tortoises took part in three experimental sessions, each lasting 10 min and conducted on separate days. At the beginning of each session, the animal was gently placed at the centre of the experimental apparatus, randomly oriented towards one of the two ends of the corridor, and then left free to explore the new environment for the entire duration of the session. In the first session, the apparatus was devoid of salient stimuli; in the second and third sessions, experimental stimuli designed to investigate amodal completion were presented at the four corners of the maze (see above). Thus subjects first underwent the stimulus-free session, followed by the two stimulus-present sessions in random order (see supplementary materials for the description of the stimuli). Each session was video-recorded using two webcams positioned above the apparatus. The videos were analysed offline using two different software tools.

For manual coding of complex behaviours, we used Boris (version 7.12.2, [48]). For each behaviour, the frequency (i.e., the number of times it occurred per trial) and duration (in seconds) were recorded, as well as its egocentric direction relative to the animal (i.e., whether it was performed towards the left or the right side of the tortoise's body).

The following behaviours were coded:

- Gaze direction: the tortoise, being inside one of the end-zones, looks toward a specific corner of the end-zone;
- Movement: the tortoise, being inside one of the end-zones, moves towards a corner;
- Proximity: the tortoise reaches touching distance of a corner, while looking at it;
- Touch: the tortoise touches a specific corner with its head and/or front legs.

The videos were also analysed using EthoVision 3.1 [49].The footage was processed at a rate of 6 frames per second, and the animals' position (x and y coordinates) was tracked using the background subtraction method. These coordinates, initially expressed in pixels, were later converted to centimetres by calibrating the software using the known width of the experimental apparatus. For analysis purposes, each hexagonal area was divided in two end-zones, a left one and a right one, while the central corridor was considered a neutral zone.

The following variables were extracted for each session:

- Visits to zone: number of times the tortoise entered each zone of the apparatus;
- Time in zone: total time spent in a specific zone (in seconds);
- Distance to corners: mean distance to each corner (in centimetres);
- Zone alternations: number of transitions made between the neutral zone and each of the two end-zones.

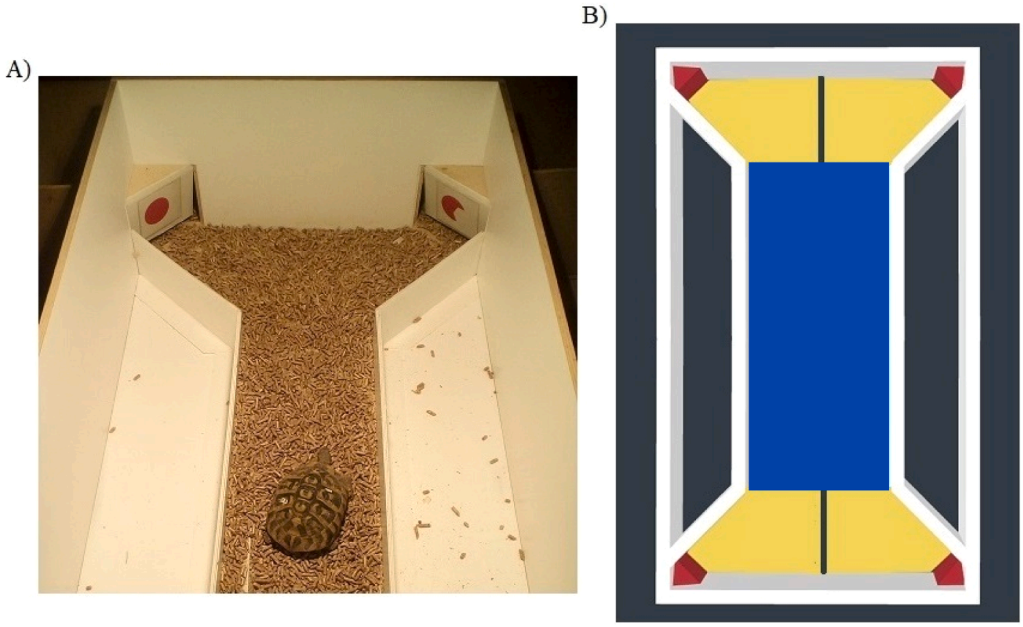


Fig. 1. (A) A female tortoise approaching one of the hexagonal areas during the second session of the test. (B) Schematic representation of the experimental apparatus, created with the softwares *Moblo 3D* and *Krita*. The hexagonal areas are marked in yellow and the two virtual end-zones used for coding are divided by a dark line. Within each end-zone, the corners of the apparatus (on which the amodal completion stimuli could be located) are highlighted in red. The neutral area is marked in blue.

3. Statistical analyses

To assess the presence of lateralization effects, a two-way mixed ANOVA was performed for each measured variable. The model included a within-subjects factor (left-right *directionality* of the behaviour) and a between-subjects factor (*sex*: males *vs.* females). Where significant interaction effects were found, paired-sample or independent-sample *t*-tests were conducted as post hoc analyses. All statistical analyses were performed using RStudio (version 4.2.1), with the significance level set at $p < 0.05$.

Specifically, for the measures coded through Boris, for each behavioural category (gaze direction, movement, proximity, and touch), frequency and duration measures were entered into a two-way mixed ANOVA with directionality (left *vs.* right) as a within-subject factor and sex (male *vs.* female) as a between-subject factor. To investigate the presence of lateralized behaviour, we conducted two complementary analyses. First, we collapsed data across all three sessions to maximise statistical power and behavioural sampling, thus obtaining a richer dataset. Second, to confirm that any observed lateralization was not influenced by the presence or configuration of visual stimuli, we repeated the same analyses on data from the first session only, in which the apparatus contained no salient cues. Consistency across these two analyses would support the robustness of the observed lateralization patterns.

Likewise, for the measures extracted through EthoVision (frequency of zone entries, total duration spent in each zone, mean distance to each corner, and number of zone alternations), each variable was entered into a two-way mixed ANOVA, with directionality (left *vs.* right end-zone) as a within-subject factor and sex (male *vs.* female) as a between-subject factor. As with the manually coded behaviours, we performed the analyses both on the collapsed dataset across all three sessions and on the first session only, to assess whether lateralization effects were evident from the very first exposure to the apparatus, in the absence of visual stimuli.

2.4. Results

3.1. Manually coded behaviours

We first analysed the Boris data collapsed ***across the three experimental sessions***. This revealed a significant main effect of *directionality* (lateralization effects) in two dependent measures reflecting, respectively, the frequency with which the animals gazed to the left and to the right corners and the duration of touches at the two corners. While animals' gaze was more often directed to the right corner ($F_{1,44} = 6.882$, $p = 0.012$, Fig. 2A), they spent more time touching the left corner ($F_{1,44} = 4.220$, $p = 0.046$, Fig. 2B).

In addition to that, a significant effect of *sex* on gaze frequency ($F_{1,44} = 15.669$, $p < 0.001$) reflected the fact that males looked at the corners more frequently overall, while the interaction *sex*directionality* was not significant ($F_{1,44} = 2.215$, $p = 0.144$). For gaze duration, no significant effects were found (*sex* $F_{1,44} = 3.155$, $p = 0.083$; *directionality* $F_{1,44} = 0.289$, $p = 0.594$; *sex*directionality* $F_{1,44} = 0.007$, $p = 0.934$). For touch, beside the above-described effect of directionality, all other effects were non-significant (touch frequency: *sex* $F_{1,44} = 3.448$, $p = 0.070$, *directionality* $F_{1,44} = 0.574$, $p = 0.453$, *sex*directionality* $F_{1,44} = 0.158$, $p = 0.693$; touch duration: *sex* $F_{1,44} = 2.754$, $p = 0.104$, *sex*directionality* $F_{1,44} = 0.037$, $p = 0.849$). For movement, both frequency and duration showed significant main effects of *sex* ($F_{1,44} = 13.441$, $p < 0.001$; $F_{1,44} = 12.913$, $p < 0.001$, respectively) and males showed more motion directed at the corners than females. No other effects reached significance (movement frequency: *directionality* $F_{1,44} = 1.404$, $p = 0.242$, *sex*directionality* $F_{1,44} = 0.408$, $p = 0.526$; movement duration: *directionality* $F_{1,44} = 1.338$, $p = 0.254$, *sex*directionality* $F_{1,44} = 0.241$, $p = 0.626$). For proximity, a significant main effect of *sex* was found for frequency ($F_{1,44} = 4.249$, $p = 0.045$), indicating that males were more frequently in proximity to the apparatus corners. All other effects were non-significant (proximity frequency: *directionality* $F_{1,44} = 0.297$, $p = 0.588$, *sex*directionality* $F_{1,44} = 0.002$, $p = 0.965$; proximity duration: *sex* $F_{1,44} = 2.886$, $p = 0.096$, *directionality* $F_{1,44} = 1.098$, $p = 0.300$, *sex*directionality* $F_{1,44} = 1.483$, $p = 0.230$). Overall, these sex effects observed indicated greater attention to exploration of the corners

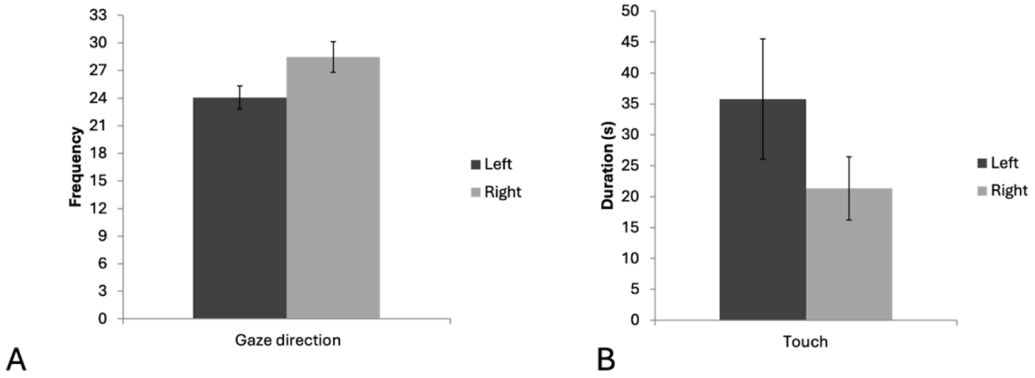


Fig. 2. Lateralised spontaneous responses in Hermann's tortoises across the three sessions of the spontaneous exploration test. (A) Mean frequency of gaze direction towards the left and right corners. Animals directed their gaze more often towards the right corner. (B) Mean duration of touch directed towards the left and right corners. Animals spent more time touching the left corner. Error bars represent SEM.

by the males.

Partially similar results emerged also from data from the ***first session only***, in which no experimental stimuli were present in the apparatus. For gaze frequency, a significant main effect of *directionality* ($F_{1,44}$ = 4.245, p = 0.045, Fig. 3) indicated a rightward looking bias.

For this measure neither the main effect of *sex* ($F_{1,44}$ = 1.789, p = 0.188) nor the *sex*directionality* interaction was significant ($F_{1,44}$ = 3.529, p = 0.067). A significant main effect of *sex* was found for gaze duration ($F_{1,44}$ = 16.633, p < 0.001), with males spending more time looking overall. No other effects were significant for gaze duration (*directionality* $F_{1,44}$ = 0.208, p = 0.650; *sex*directionality* $F_{1,44}$ = 0.016, p = 0.899). For touch, a significant main effect of *sex* was found in the frequency ($F_{1,44}$ = 4.756, p = 0.035) and duration ($F_{1,44}$ = 4.533, p = 0.039), with this behaviour being more pronounced in males. No significant *directionality* effect was found (touch frequency: $F_{1,44}$ = 0.588, p = 0.447; touch duration: $F_{1,44}$ = 2.011, p = 0.163), nor any significant *sex*directionality* interactions emerged (touch frequency: $F_{1,44}$ = 2.377, p = 0.130; touch duration: $F_{1,44}$ = 0.0005, p = 0.982). For proximity frequency and proximity duration, significant main effects of sex were also found ($F_{1,44}$ = 4.614, p = 0.037; $F_{1,44}$ = 6.765, p = 0.013, respectively), with males showing closer proximity to the corners than females. For proximity frequency, neither *directionality* effect ($F_{1,44}$ = 0.211, p = 0.648), nor the *sex*directionality* interaction ($F_{1,44}$ = 1.130, p = 0.294) reached significance, nor did any other effects for duration (*directionality* $F_{1,44}$ = 0.415, p = 0.523; *sex*directionality* $F_{1,44}$ = 0.076, p = 0.784). For movement, significant main effects of *sex* were found for both frequency ($F_{1,44}$ = 10.116, p = 0.003) and duration ($F_{1,44}$ = 21.942, p < 0.001), with males again displaying more locomotor activity. No significant effects of *directionality* (movement frequency: $F_{1,44}$ = 0.115, p = 0.737; movement duration: $F_{1,44}$ = 0.087, p = 0.770) or *sex*directionality* interactions (movement frequency: $F_{1,44}$ = 1.324, p = 0.256; movement duration: $F_{1,44}$ = 0.0007, p = 0.979) were found. To assess whether the observed lateralization patterns were stable across sessions, we ran an additional mixed ANOVA including session (three levels, within-subject) as an additional factor alongside sex and directionality. This analysis revealed no significant interactions between directionality and session, indicating that the lateralization profile remained consistent throughout the test. Full statistical results are reported in Supplementary Table 1.

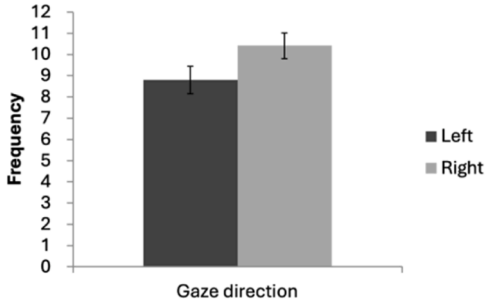


Fig. 3. Lateralization in the mean frequency of gaze direction towards the left and the right corners, exhibited by the tortoises during the first session. Error bars represent SEM.

Summarising, in terms of lateralization, an intriguing dissociation emerged between increased gazing towards the right side of space and increasing physical exploration towards the left, as revealed by touch duration, when data were analysed across all test sessions. This pattern was partially confirmed when analyses were limited to the first test session only (in the absence of any salient stimuli on the corners), which revealed increased gazing towards the right corner already at this stage, and did not appear to change during the course of the test. Moreover, regardless of lateralization, sex effects indicated greater locomotor activity in males, as well as visual attention and physical exploration of the end-zones of the apparatus.

3.2. Automated measurements

We then analysed the measures extracted through EthoVision, collapsed ***across the three experimental sessions***. A significant main effect of *directionality* emerged for both the number of visits to each end-zone ($F_{1,44}$ = 50.110, p < 0.001) and the number of transitions between the neutral and end-zones ($F_{1,44}$ = 17.975, p < 0.001), indicating that tortoises displayed lateralised spatial preferences in their exploratory patterns. Importantly, *sex*directionality* interactions were significant for both zone entries ($F_{1,44}$ = 42.658, p < 0.001) and alternations ($F_{1,44}$ = 35.919, p < 0.001). Post hoc *t*-tests revealed that these effects were driven by males, who visited the left zone significantly more often than the right (t_{23} = 7.726, p < 0.001, Fig. 4A), but made more transitions between the neutral zone and the right side (t_{23} = −6.8703, p < 0.001, Fig. 4B), while no lateralised pattern was observed in females (zone visits: t_{21} = 0.6715, p = 0.509; alternations: t_{21} = 0.7399, p = 0.468, Figs. 4A and 4B).

For the time spent in each zone, too, a significant main effect of *directionality* ($F_{1,44}$ = 7.986, p = 0.007) was found, indicating that animals, regardless of sex, spent more time on the left side of the apparatus (Fig. 5). The interaction between sex and directionality, however, did not reach significance ($F_{1,44}$ = 1.656, p = 0.205).

Additionally, a main effect of *sex* was found for both zone entries ($F_{1,44}$ = 24.266, p < 0.001) and alternations ($F_{1,44}$ = 26.814, p < 0.001), with males generally being more active than females. For the time spent in each zone, a significant main effect of *sex* ($F_{1,44}$ = 25.658, p < 0.001) indicated that males spent more time in the end-zones compared to females. Likewise, for the average distance to the

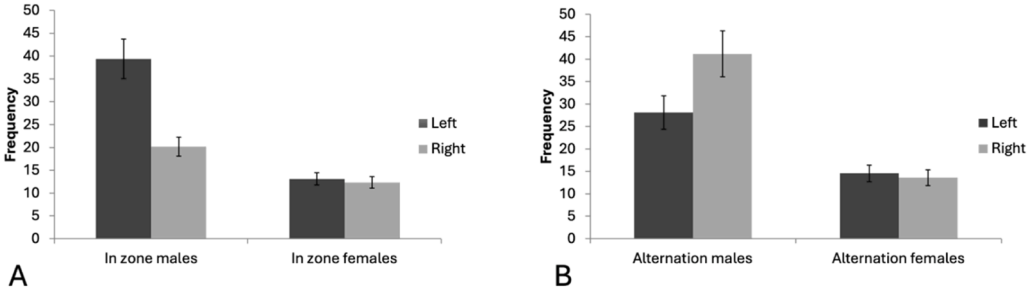


Fig. 4. Lateralised spontaneous responses in Hermann's tortoises across the three sessions of the spontaneous exploration test. (A) Mean frequency of occupancy of the right and left choice zones and in males and females. (B) Mean frequency of alternations between the neutral zone and each choice zone in the two sexes. Error bars represent SEM.

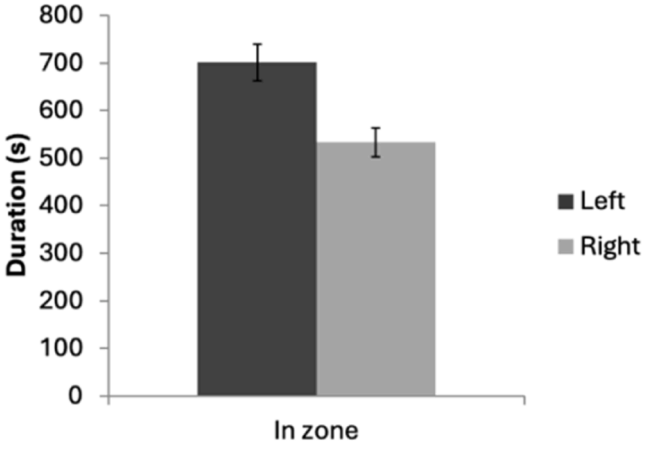


Fig. 5. Lateralization in the average time (in seconds) spent in the left and right end zones by the tortoises, across the three sessions. Error bars represent SEM.

zone corners, a main effect of *sex* was observed ($F_{1,44}$ = 890.275, $p < 0.001$). Both these effects reflect the fact that males tended to stay closer to the corners compared to females, in line with what emerged from the manually coded measures. Neither the main effect of *directionality* ($F_{1,44} = 2.375$, $p = 0.130$) nor the *sex*directionality* interaction ($F_{1,44} = 0.109$, $p = 0.742$) were statistically significant for this variable.

We finally analysed EthoVision-derived behavioural data from the ***first test session only***, when no experimental stimuli were present in the apparatus, revealing a very similar pattern of results. For zone entries, a significant main effect of *directionality* ($F_{1,44} = 39.256$, $p < 0.001$) and of *sex* ($F_{1,44}$ = 12.035, $p < 0.001$) emerged. Crucially, a significant *sex*-directionality* interaction was found ($F_{1,44} = 37.529$, $p < 0.001$, Fig. 6A). Post hoc comparisons indicated that only males showed a lateralised pattern of zone visits, entering the left zone significantly more often than the right (t_{23} = 7.1282, $p < 0.001$), while females showed no such preference ($t_{21} = 0.1596$, $p = 0.875$). For zone alternations, once again, a significant effect of *directionality* ($F_{1,44} = 19.580$, $p < 0.001$), *sex* ($F_{1,44}$ = 10.540, $p = 0.002$), and a *sex*directionality* interaction ($F_{1,44}$ = 24.558, $p < 0.001$) were detected. Follow-up tests confirmed that males alternated more frequently between the neutral zone and the right side ($t_{23} = -5.7865$, $p < 0.001$), while no lateralised alternation pattern was present in females ($t_{21} = 0.4779$, $p = 0.638$) (Fig. 6B).

For the time spent in each zone, a main effect of *sex* was found ($F_{1,44}$ = 6.305, $p = 0.016$), but neither the effect of *directionality* ($F_{1,44}$ = 3.059, $p = 0.087$) nor the *sex*directionality* interaction ($F_{1,44} = 0.319$, $p = 0.575$) reached significance. Regarding distance to the corners, the analysis again revealed a main effect of *sex* ($F_{1,44} = 805.484$, $p < 0.001$), with males staying closer to the corners overall. No significant effect of *directionality* ($F_{1,44} = 1.038$, $p = 0.314$) or *sex*directionality* interaction ($F_{1,44} = 0.396$, $p = 0.533$) was observed. Also in this case, to assess the stability of the observed lateralization patterns, we ran an additional ANOVA including session as a factor. Once again, this revealed no significant interactions between directionality and session. Full statistical results are reported in Supplementary Table 2.

To sum up, also for the data collected through automated tracking, we observed a dissociation in the direction of lateralization effects revealed by different measures. Once again, while animals overall spent more time exploring the left end-zones (as revealed by data collapsed across all sessions), regardless of the session males alternated more frequently between the neutral zone and the right end-zone. None of these lateralization effects revealed a significant change across sessions. This suggests a more cautious exploratory strategy in that spatial direction—moving back and forth rather than committing to full entry. Finally, sex effects too are consistent with the manually coded behaviours, with males displaying overall higher activity levels and increased exploration of the end zones and apparatus corners.

4. Discussion

The main aim of this study was to investigate the presence and direction of behavioural lateralization in *Testudo hermanni* during spontaneous exploration in a novel environment. This allowed us to verify

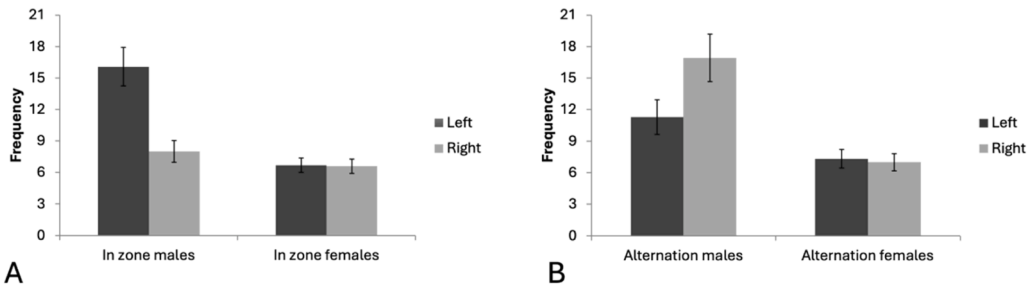


Fig. 6. Lateralised spontaneous responses in Hermann's tortoises in the first session. (A) Mean frequency of occupancy of the right and left choice zones and in males and females. (B) Mean frequency of alternations between the neutral zone and each choice zone in the two sexes. Error bars represent SEM.

whether tortoises would exhibit lateralized behaviour consistent with previous findings (e.g., [38,41]), while describing the spontaneous behavioural patterns that emerged in a novel, controlled setting (a semi-naturalistic context and using a non-appetitive, spontaneous exploration task). In addition, we wanted to explore whether lateralization was modulated by sex (i.e., whether males and females would differ in the strength or direction of lateralized behaviours). Finally, taking advantage of the fact that this study was part of a broader investigation on amodal completion percepts (not yet published), which involved multiple test sections in the presence/absence of multiple stimuli, we could also test whether asymmetries observed in early exposure (stimulus-free) would persist or change when stimuli were later introduced. This allowed us to investigate the stability of the observed lateralization patterns, starting to shed light on whether they might be stable and trait-like, or instead context-dependent and sensitive to external cues.

Overall, our results revealed a lateralization pattern characterised by a dissociation across groups of dependent measures. A leftward bias emerged in variables likely reflecting active exploration and direct approach behaviour. Specifically, tortoises showed longer touch duration toward the left corner (when data were pooled across sessions) and, in males, a higher number of entries into the left zone. In contrast, a rightward bias was observed in measures such as increased gazing toward the right side and, in males, more frequent alternations between the neutral zone and the right end-zone. This pattern may reflect a more cautious or hesitant engagement with the right side—characterised by repeated monitoring or probing without committing to full entry. This dissociation may be interpreted in light of Andrew's [32] global theory of hemispheric specialization. According to this framework, the left-eye system (right hemisphere) is specialized for rapid readiness to respond to releaser stimuli. Its attention is easily captured by salient or novel cues, and it tends to promote relatively impulsive approach behaviour. In the present case, this could be reflected in the greater tendency to enter the end-zones and directly touch the corners. By contrast, the right-eye system (left hemisphere) is specialized for sustained attention and visuomotor control. This includes response inhibition, action planning, and the filtering of inappropriate responses. It is also associated with detailed inspection of familiar stimuli and with regulating approach behaviour under conditions of uncertainty. In our data, this may be expressed as repeated, cautious checking of one side rather than full commitment to exploration. A brief qualification is worth noting. In this framework, the right hemisphere is not exclusively linked to approach behaviour; it also supports vigilance and rapid defensive responses. Thus, "impulsive" here should be understood as fast and stimulus-driven, which may result in either approach or withdrawal depending on the context. Hemispheric differences are thus task-dependent: in learned, rule-based paradigms (e.g., Go/No-Go tasks), the left hemisphere can appear faster than the right, but this reflects top-down control rather than impulsivity (e.g., [50,51]).

The presence of lateralization is in line with previous evidence showing behavioural asymmetries in tortoises or other chelonians. For instance, Hermann's tortoises have shown asymmetric paw- and eye-use in the presence of social vs. neutral stimuli ([41], see also [38]). Specifically, they display a left-eye preference when inspecting their reflection in a mirror, suggesting right-hemisphere involvement in processing information from conspecifics. In contrast, they show a right-eye bias in the absence of mirrors, when exploring a novel, neutral, and homogeneous environment. In this latter context, the left hemisphere appears to support inspection and decision-making about where to move while categorising a new experience [41]. Further evidence that behavioural lateralization in Hermann's tortoises may depend on the specific task comes from a recent study investigating discrimination performance in this species [52]. In a task requiring discrimination between openings of different widths, individual lateralization biases were relatively consistent across testing sessions, despite the absence of a significant population-level directional bias when all trials were considered. Interestingly, a significant leftward bias emerged when only incorrect responses were analysed. These findings suggest that individual side preferences may remain consistent even when no uniform directional bias is evident at the population level, and that the expression of lateralization may vary with task demands. This is relevant to the present findings, in which rightward attentional biases co-occurred with leftward exploratory behaviours, highlighting the importance of distinguishing between behavioural components when investigating lateralization in chelonians. Converging evidence from other chelonian species further supports the presence of lateralized behaviours, aligned with the general pattern of hemispheric specialization outlined by Andrew [32]. Aldabra giant tortoises show a right-head resting posture (with the left eye outward), consistent with preferential right-hemisphere involvement in vigilance and social-threat monitoring [44]. Similarly, European pond turtles (*Emys orbicularis*) display a left-side preference in escape and righting responses in antipredator contexts [45].

Comparatively little is known about the neural basis of cerebral lateralization in chelonians, and in tortoises in particular. However, one crucial aspect is comparatively well characterised: the way in which visual information from each eye is distributed between the two sides of the brain. Although direct evidence from Testudo is limited, in the painted turtle *Chrysemys picta* most retinal fibres decussate at the optic chiasm, with a minor ipsilateral component [53]. Comparative work across 21 chelonian species further indicates that contralateral retinal projections are a highly conserved feature of the primary visual system, whereas ipsilateral projections vary considerably among species [54]. A similar organization has been described in other reptiles, suggesting that this may represent a conserved feature [55,56]. Visual information can also undergo further bilateral redistribution at later processing stages. Within Testudo, tectothalamic projections have been directly traced only in T. horsfieldii, where the major projections described were ipsilateral [57]. Secondary crossing of tectal output has, however, been demonstrated in another chelonian, Pseudemys scripta, and in lizards, where tectal projections cross through the supraoptic decussation to reach the contralateral diencephalon [58,59]. By contrast, available tract-tracing studies of the chelonian thalamofugal pathway describe ipsilateral thalamocortical projections, without providing evidence for a comparable secondary crossing [60]. Finally, the two telencephalic hemispheres are themselves interconnected: for instance in *Testudo horsfieldi* and *Emys orbicularis*, dorsal-cortical efferents have been traced into the hippocampal commissure [61], while direct interhemispheric pallial projections through commissural systems have also been demonstrated in other reptiles [62]. Thus, as in other vertebrates with laterally placed eyes, it is reasonable to assume that most of the visual information guiding behaviour preferentially reaches the hemisphere contralateral to the eye in use, while some information could also reach the ipsilateral hemisphere.

Whether the main visual pathways conveying information from each eye differ systematically between the left and right sides remains largely unknown in reptiles. Nevertheless, other forms of neural asymmetry are known. For instance, the turtle visual cortex shows side-specific neurochemical regulation, with left and right cortices differing in both the effects and binding of different opioid receptor agonists [63]. Developmental asymmetries have also been demonstrated in chelonians, including left-sided expression of the Nodal–Lefty–Pitx2 signalling module in the embryonic forebrain and asymmetric habenular gene expression [64]. In lizards, structural and connectional asymmetry of the habenular system has also been demonstrated, including a unilateral projection from the parietal eye to the left medial habenula [65]. Thus, while the neural substrates underlying hemispheric functional specialization in chelonians remain largely unknown, the available evidence demonstrates that left–right differentiation is present at functional, neurochemical, structural and developmental levels in the reptilian brain.

At the behavioural level, in our results, we can observe that some

lateralization effects proved robust, as they emerged both in the pooled dataset (across all sessions) and in the first session alone, when the apparatus was free of stimuli. These included a rightward bias in gaze direction, with animals looking more often toward the right corner, from their very first exposure to the apparatus. In addition, a male-specific spatial asymmetry was observed in exploratory behaviour: males entered the left end-zone more frequently and showed more alternations between the neutral zone and the right end-zone. Other lateralization effects emerged only when data were pooled across sessions, suggesting less stable patterns. In particular, tortoises spent longer time in the left zone and showed a leftward bias in touch duration only in the combined dataset. Thus, at least for some measures, this lateralization pattern appeared to be a relatively stable phenomenon, present across the repeated test-sessions conducted in the same individuals across three different days.

Moreover, some of the lateralization effects we observed were limited to males (higher number of entries in the left zone and more frequent alternations between the neutral zone and the right end-zone). Sex-specific patterns of asymmetry are not unique to our findings and have been observed in other reptile taxa. For instance, in water turtles, a leftward bias in righting responses has been observed, and this asymmetry is markedly stronger in males, possibly in relation to the well-known sexual dimorphism in shell shape [66]. In a territorial lizard species, leftward turning biases appear more pronounced in males, potentially reflecting their stronger territorial tendencies [37]. By contrast, in a non-territorial, viviparous lizard species, only females show a leftward turning bias during maze exploration [36], suggesting that direction and expression of dimorphic asymmetries may reflect species-specific ecological or behavioural adaptations.

Sexually dimorphic lateralization is not restricted to reptiles and has been reported in birds (e.g., [67–69]), in fish (e.g., [70,71]) and in mammals, (e.g., [72,73]) suggesting that sex hormones may contribute to the development or modulation of several forms of lateralization (e.g., [69,74–79], see [80], for a recent review on sex hormones and human handedness).

Beyond lateralization effects, we also observed a clear sex difference in overall motility, with males showing higher levels of movement than females. This finding is consistent with the marked morphological sexual dimorphism characterising this species. Male shells are shaped in a way that may facilitate greater agility and faster locomotion [81], possibly reflecting selection pressures related to mate search, mate pursuit—given that females often move away from courting males—and the performance of courtship displays. Alternatively, the female body plan may have been shaped by selection for fecundity, allowing females to carry a larger number of eggs at the expense of motility [82].

Lower mobility in females could also reflect differences in behavioural regulation. In the same species, females have been reported to show higher inhibitory control [83], which might contribute to reduced exploratory movement. Consistent with our findings, greater mobility in males has also been documented in righting-response studies in water turtles [66].

The present study presents relevant methodological strengths. First, there was a good correspondence between manually coded behaviours and automatically extracted tracking measures, supporting the robustness and internal consistency of the results. The convergence between these two independent coding approaches strengthens confidence in the reliability of the observed patterns.

An important feature of the present study is the use of a semi-naturalistic testing environment and a non-appetitive, spontaneous exploration task. Unlike forced-choice paradigms or reward-based discrimination tasks, which may introduce strong motivational biases or stress-related effects, our approach allowed for the observation of naturalistic, low-stress behaviours that better reflect the animal's intrinsic tendencies. The tortoises were free to explore the apparatus without being trained, handled repeatedly, or required to perform specific actions to obtain reinforcement. This ecological setup enhances the external validity of the findings and supports the idea that the observed lateralization patterns likely reflect spontaneous, trait-like biases rather than task-induced asymmetries. Moreover, conducting the experiment in a semi-naturalistic context—within the SperimentArea facility where the animals were housed—minimised environmental novelty and maintained continuity with the tortoises' daily living conditions. This may have facilitated more stable and reliable behavioural expressions, particularly in a species that is typically solitary and slow to adapt to abrupt changes. Together, these methodological choices contribute to the strength of the present findings and offer a valuable model for studying lateralization in reptiles under conditions that are both ethologically valid and experimentally controlled.

The present study also has several limitations that should be acknowledged. First, its exploratory nature calls for caution in generalising the findings. Some behavioural variables, such as touch and gaze direction toward the corners, were manually coded and may therefore be subject to observer bias. However, the overall correspondence between manually coded and automatically extracted measures suggests that manual scoring was reliable and did not fundamentally alter the pattern of results.

In addition, the order of test sessions was not fully counterbalanced. All subjects first underwent the stimulus-free session, followed by the two stimulus-present sessions in random order. This design introduces a potential confound, as differences between the first and subsequent sessions could reflect novelty, habituation, or fatigue effects. Nevertheless, the general pattern of lateralization was largely consistent between the first session and the pooled data, which reduces the likelihood that session order critically shaped the main findings. Finally, caution is warranted in interpreting the interaction between sex and lateralization. Because males showed higher overall activity levels, differences in motor output may have amplified the apparent strength of lateralised behaviours in this group. Sex-related asymmetries should therefore be interpreted carefully, as they may partly reflect differences in general motility rather than purely lateralised processes.

5. Conclusions

In conclusion, the present study provides evidence for the presence of behavioural lateralization in *Testudo hermanni* during spontaneous exploration in a semi-naturalistic environment. The observed pattern, characterised by a dissociation between leftward biases in active approach behaviours and rightward biases in monitoring-related responses, is broadly consistent with current frameworks of hemispheric specialization. Importantly, some of these asymmetries appeared stable across sessions, suggesting that they may reflect intrinsic, trait-like tendencies rather than transient, context-dependent effects. At the same time, the modulation of certain behaviours by sex highlights the potential influence of biological and ecological factors on the expression of lateralization. By adopting a non-appetitive, low-stress paradigm, this study contributes to a growing body of research emphasising the value of ecologically valid approaches in the investigation of animal cognition and behaviour. However, given the exploratory nature of the study and the presence of some methodological constraints, further research is needed to confirm and extend these findings. Future studies should aim to disentangle the role of motivational, contextual, and physiological variables, as well as to clarify the functional significance of the observed asymmetries.

Overall, these findings support the view that lateralization is a robust and evolutionarily widespread feature of vertebrate brains, and they highlight the relevance of studying it in under-investigated taxa such as chelonians under naturalistic conditions.

CRediT authorship contribution statement

Orsola Rosa-Salva: Writing – review & editing, Writing – original draft, Supervision, Formal analysis, Data curation, Conceptualization.

Emanuela Musto: Writing – review & editing, Investigation, Formal analysis, Data curation. **Anastasia Morandi-Raikova:** Writing – review & editing, Formal analysis, Data curation. **Maria Elena Miletto-Petrazzini:** Writing – review & editing, Supervision, Conceptualization. **Gionata Stancher:** Writing – review & editing, Resources. **Angelo Bisazza:** Writing – review & editing, Conceptualization. **Valeria Anna Sovrano:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used generative AI (ChatGPT) for improving text readability. The authors reviewed and edited the output as needed and take full responsibility for the content of the published article.

Funding

The present study was supported by intramural resources to V.A.S., from the Center for Mind/Brain Sciences (CIMeC, University of Trento), and to G.S., from the Civic Museum Foundation of Rovereto (MCR Foundation, Trento, Italy).

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgments

The authors are thankful to Greta Baratti and Tommaso Pecchia for their technical support with videos, apparatus, stimuli, and the marking and identification of animals, and to Daniele Musto for his assistance in creating the schematic representation of the experimental apparatus.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bbr.2026.116556.

Data Availability

Raw Data available in supplementary materials.

References

[1] A. Bisazza, C. Cantalupo, A. Robins, L.J. Rogers, G. Vallortigara, Right-pawedness in toads, Nature 379 (1996) 408.
[2] A. Bisazza, L.J. Rogers, G. Vallortigara, The origins of cerebral asymmetry: a review of evidence of behavioural and brain lateralization in fishes, reptiles and amphibians, Neurosci. Biobehav. Rev. 22 (1998) 411–426.
[3] E. Frasnelli, G. Vallortigara, L.J. Rogers, Left-right asymmetries of behaviour and nervous system in invertebrates, Neurosci. Biobehav. Rev. 36 (2012) 1273–1291.
[4] M.E. Miletto Petrazzini, V.A. Sovrano, G. Vallortigara, A. Messina, Brain and Behavioral Asymmetry: A Lesson From Fish, Front. Neuroanat. 26 (14) (2020) 11.
[5] L.J. Rogers, R.J. Andrew, Comparative vertebrate lateralization, Cambridge University Press, New York, 2002.
[6] L.J. Rogers, G. Vallortigara, R.J. Andrew, Divided brains: the biology and behaviour of brain asymmetries, Cambridge University Press, 2013.
[7] G. Vallortigara, L.J. Rogers, Survival with an asymmetrical brain: advantages of cerebral lateralization, Behav. Brain Sci. 28 (2005) 575–589.
[8] G. Vallortigara, L.J. Rogers, A. Bisazza, Possible evolutionary origins of cognitive brain lateralization, Brain Res. Rev. 30 (1999) 164–175.
[9] A. Bisazza, T. Lucon-Xiccato, Individual differences in vertebrate behavioural lateralisation: the role of genes and environment, Symmetry 17 (2025) 527.
[10] J. Levy, The mammalian brain and the adaptive advantage of cerebral asymmetry, Ann. N. Y. Acad. Sci. 299 (1977) 264–272.
[11] C. Cantalupo, A. Bisazza, G. Vallortigara, Lateralization of predator-evasion response in a teleost fish (Girardinus falcatus), Neuropsychologia 33 (1995) 1637–1646.
[12] R.J. Andrew, The nature of behavioural lateralization in the chick, in: R.J. Andrew (Ed.), Neural and Behavioural Plasticity: The Use of the Domestic Chick as a Model, Oxford Science Publications, Oxford, 1991, pp. 536–554.
[13] G. Vallortigara, The Evolutionary Psychology of Left and Right: Costs and Benefits of Lateralization, Dev. Psychobiol. 48 (2006) 418–427.
[14] M. Dadda, A. Bisazza, Does brain asymmetry allow efficient performance of simultaneous tasks? Anim. Behav. 72 (2006) 523–529.
[15] L.J. Rogers, Evolution of hemispheric specialization: advantages and disadvantages, Brain Lang. 15 (73) (2000) 236–253.
[16] E. Frasnelli, G. Vallortigara, Individual-Level and Population-Level Lateralization: Two Sides of the Same Coin, Symmetry 10 (2018) 739.
[17] A. Bisazza, C. Cantalupo, M. Capocchiano, G. Vallortigara, Population lateralisation and social behavior: A study with 16 species of fish, Laterality 5 (2000) 269–284.
[18] A. Bisazza, M. Dadda, Enhanced schooling performance in lateralized fishes, Proc. Biol. Sci. 272 (2005) 1677–1681.
[19] S. Ghirlanda, G. Vallortigara, The evolution of brain lateralization: a game-theoretical analysis of population structure, Proc. Biol. Sci. 271 (2004) 853–857.
[20] N.P. Austin, L.J. Rogers, Asymmetry of flight and escape turning responses in horses, Laterality 12 (2007) 464–474.
[21] B. Bonati, D. Csermely, P. López, J. Martín, Lateralization in the escape behaviour of the common wall lizard (Podarcis muralis), Behav. Brain Res. 207 (2010) 1–6.
[22] B. Bonati, D. Csermely, V.A. Sovrano, Looking at a predator with the left or right eye: asymmetry of response in lizards, Laterality 18 (2013) 329–339.
[23] O. Güntürkün, S. Kesch, Visual lateralization during feeding in pigeons, Behav. Neurosci. 101 (1987) 433–435.
[24] O. Güntürkün, B. Diekamp, M. Manns, F. Nottelmann, H. Prior, A. Schwarz, M. Skiba, Asymmetry pays: visual lateralization improves discrimination success in pigeons, Curr. Biol. 10 (2000) 1079–1081.
[25] G. Lippolis, A. Bisazza, L.J. Rogers, G. Vallortigara, Lateralisation of predator avoidance responses in three species of toads, Laterality 7 (2002) 163–183.
[26] G. Lippolis, W. Westman, B.M. McAllan, L.J. Rogers, Lateralisation of escape responses in the stripe-faced dunnart, Sminthopsis macroura (Dasyuridae: Marsupialia), Laterality 10 (2005) 457–470.
[27] J.A. Mench, R.J. Andrew, Lateralization of a food search task in the domestic chick, Behav. Neural Biol. 46 (1986) 107–114.
[28] A. Robins, L.J. Rogers, Lateralized prey-catching responses in the cane toad, Bufo marinus: Analysis of complex visual stimuli, Anim. Behav. 68 (2004) 767–775.
[29] V.A. Sovrano, Visual lateralization in response to familiar and unfamiliar stimuli in fish, Behav. Brain Res. 9 (152) (2004) 385–391.
[30] V.A. Sovrano, R.J. Andrew, Eye use during viewing a reflection: behavioural lateralisation in zebrafish larvae, Behav. Brain Res. 28 (167) (2006) 226–231.
[31] G. Vallortigara, L.J. Rogers, A. Bisazza, G. Lippolis, A. Robins, Complementary right and left hemifield use for predatory and agonistic behaviour in toads, NeuroReport 9 (1998) 3341–3344.
[32] R.J. Andrew, Origins of asymmetry in the CNS, Semin. Cell. Dev. Biol. 20 (2009) 485–490.
[33] A. Robins, P. Chen, L. Beazley, S. Dunlop, Lateralized predatory responses in the ornate dragon lizard (Ctenophorus ornatus), NeuroReport 16 (2005) 849–852.
[34] B. Bonati, D. Csermely, R. Romani, Lateralization in the predatory behaviour of the common wall lizard (Podarcis muralis), Behav. Process. 79 (2008) 171–174.
[35] J. Martín, P. López, B. Bonati, D. Csermely, Lateralization When Monitoring Predators in the Wild: A Left Eye Control in the Common Wall Lizard (Podarcis muralis), Ethology 116 (2010) 1226–1233.
[36] B. Bonati, C. Quaresmini, G. Stancher, V.A. Sovrano, How ecology could affect cerebral lateralization for explorative behaviour in lizards, Symmetry 9 (2017) 1–9.
[37] D. Csermely, B. Bonati, P. López, J. Martín, Is the Podarcis muralis lizard left-eye lateralised when exploring a new environment? Laterality 16 (2011) 240–255.
[38] G. Stancher, E. Clara, L. Regolin, G. Vallortigara, Lateralized righting behavior in the tortoise (Testudo hermanni), Behav. Brain Res. 173 (2006) 315–319.
[39] Y. Malashichev, Asymmetry of righting reflexes in sea turtles and its behavioural correlates, Physiol. Behav. 157 (2016) 1–8.
[40] G.R. Smith, J.E. Rettig, J.B. Iverson, Is righting response lateralized in two species of freshwater turtles? Behaviour 154 (2017) 1069–1079.
[41] V.A. Sovrano, C. Quaresmini, G. Stancher, Tortoises in front of mirrors: Brain asymmetries and lateralized behaviours in the tortoise (Testudo hermanni), Behav. Brain Res. 15 (352) (2018) 183–186, https://doi.org/10.1016/j.bbr.2017.06.021.
[42] O. Rosa-Salva, L. Regolin, E. Mascalzoni, G. Vallortigara, Cerebral and behavioural asymmetries in animal social recognition, Comp. Cogn. Behav. Rev. 7 (2012) 110–138.
[43] R.J. Davidson, Well–being and affective style: neural substrates and biobehavioural correlates, Philos. Trans. Roy. Soc. B. 359 (2004) 1395–1411.
[44] C. Spiezio, C. Sandri, F. Joubert, M.M. Muzungaile, S. Remy, P. Mattarelli, B. Regaiolli, The "right" side of sleeping: laterality in resting behaviour of Aldabra giant tortoises (Aldabrachelys gigantea), Anim. Cogn. 25 (2022) 195–203.
[45] D. Pellitteri-Rosa, A. Gazzola, Context-dependent behavioural lateralization in the European pond turtle, Emys orbicularis (Testudines, Emydidae), J. Exp. Biol. 221 (2018).
[46] S.J. Girling, Veterinary nursing of exotic pets, John Wiley & Sons, 2008.
[47] G. Baratti, M. Luongo, M. Ponticorvo, T. Pecchia, G. Stancher, O. Gigliotta, V. A. Sovrano, Navigation and spatial learning in Hermann's tortoises: Insight from geometric reorientation, J. Exp. Psychol. Anim. Lear. Cogn. (2026) 1–27, https://doi.org/10.1037/xan0000423.

[48] O. Friard, M. Gamba, BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations, Methods Ecol. Evol. 7 (2016) 1325–1330.
[49] L.P. Noldus, A.J. Spink, R.A. Tegelenbosch, EthoVision: a versatile video tracking system for automation of behavioral experiments, Behav. Res. Methods Instrum. Comput. 33 (2001) 398–414.
[50] S. Hirose, J. Chikazoe, T. Watanabe, K. Jimura, A. Kunimatsu, O. Abe, K. Ohtomo, Y. Miyashita, S. Konishi, Efficiency of go/no-go task performance implemented in the left hemisphere, J. Neurosci. 32 (2012) 9059–9065.
[51] G. Vallar, E. Bisiach, M. Cerizza, M.L. Rusconi, The role of the left hemisphere in decision-making, Cortex 24 (1988) 399–410.
[52] A. Bisazza, E. Gatto, E. De Pizzol, G. Stancher, V.A. Sovrano, No evidence of body size awareness in Hermann's tortoise (Testudo hermanni), Curr. Zool. (2026) (2026) 1–15, zoag043.
[53] A.H. Bass, R.G. Northcutt, Retinal recipient nuclei in the painted turtle, Chrysemys picta: An autoradiographic and HRP study, J. Comp. Neurol. 199 (1981) 97–112.
[54] S. Hergueta, M. Lemire, R. Ward, J. Repérant, J.P. Rio, C. Weidner, Interspecific variation in the chelonian primary visual system, J. Hirnforsch. 36 (1995) 171–193.
[55] G. Casini, P. Petrini, A. Foà, P. Bagnoli, Pattern of organization of primary visual pathways in the European lizard Podarcis sicula Rafinesque, J. Hirnforsch. 34 (1993) 361–374.
[56] J. Repérant, J.P. Rio, D. Miceli, M. Lemire, A radioautographic study of retinal projections in type I and type II lizards, Brain Res. 142 (1978) 401–411.
[57] M. Belekhova, N. Kenigfest, J.P. Rio, J. Repérant, R. Ward, N. Vesselkin, O. Karamian, Tectothalamic visual projections in turtles: Their cells of origin revealed by tracing methods, J. Comp. Neurol. 457 (2003) 37–56.
[58] S.O.E. Ebbesson, Projections of the optic tectum and the mesencephalic nucleus of the trigeminal nerve in the tegu lizard (Tupinambis nigropunctatus), Cell. Tissue Res. 216 (1981) 151–165.
[59] R.E. Foster, W.C. Hall, The connections and laminar organization of the optic tectum in a reptile (Iguana iguana), J. Comp. Neurol. 163 (1975) 397–425.
[60] D. Zhu, K.H. Lustig, K. Bifulco, J. Keifer, Thalamocortical connections in the pond turtle Pseudemys scripta elegans, Brain Behav. Evol. 65 (2005) 278–292.
[61] G.V. Gaidaenko, Descending connections of the dorsal cortex in turtles, Zh. Evol. Biokhim. Fiziol. 13 (1977) 416–418.
[62] F. Martínez-García, M. Amiguet, W.K. Schwerdtfeger, F.E. Olucha, M.J. Lorente, Interhemispheric connections through the pallial commissures in the brain of Podarcis hispanica and Gallotia stehlinii (Reptilia, Lacertidae), J. Morphol. 205 (1990) 17–31.
[63] G.Y. Bakalkin, A.S. Pivovarov, A.G. Kobylyansky, P.N. Nesterenko, K.N. Yarygin, Lateralization of opioid receptors in turtle visual cortex, Brain Res. 480 (1889) 268–276. https://www.sciencedirect.com/science/article/abs/pii/0006899389901923?via%3Dihub.
[64] E. Kajikawa, T. Miki, M. Takeda, H. Kiyonari, H. Hamada, Left-right asymmetric expression of the Nodal-Lefty-Pitx2 module in developing turtle forebrain, Front. Cell Dev. Biol. 10 (2022) 929808, https://doi.org/10.3389/fcell.2022.929808.
[65] G.A. Engbretson, A. Reiner, N. Brecha, Habenular asymmetry and the central connections of the parietal eye of the lizard, J. Comp. Neurol. 198 (1) (1981) 155–165, https://doi.org/10.1002/cne.901980113.
[66] D. Pellitteri-Rosa, M. Lazić, A. Gazzola, G. Vallortigara, Righting behaviour in the European pond turtle (Emys orbicularis): relations between behavioural and morphological lateralization, Anim. Cogn. 23 (2020) 989–998.
[67] L.J. Rogers, Early experiential effects on laterality: research on chicks has relevance to other species, Laterality 2 (1997) 199–219.
[68] M. Romano, M. Parolini, M. Caprioli, C. Spiezio, D. Rubolini, N. Saino, Individual and population-level sex-dependent lateralization in yellow-legged gull (Larus michahellis) chicks, Behav. Process. 115 (2015) 109–116, https://doi.org/10.1016/j.beproc.2015.03.012.
[69] J. Wade, L. Buhlman, Lateralization and effects of adult androgen in a sexually dimorphic neuromuscular system control- ling song in zebra finches, J. Comp. Neurol. 426 (2001) 154–164.
[70] A.L. Bibost, C. Brown, Laterality influences schooling position in rainbowfish, Melanotaenia spp, PLoS. ONE 8 (2013) 1–8.
[71] A.R. Reddon, P.L. Hurd, Sex differences in the cerebral lateralisation of a cichlid fish when detouring to view emotionally conditioned stimuli, Behav. Process. 82 (2009) 25–29.
[72] J. Alonso, M.A. Castellano, M. Rodriquez, Behavioral lateralisation in rats: prenatal stress effects on sex differences, Brain Res. 539 (1991) 45–50.
[73] G. Tabibnia, B.M. Cooke, S.M. Breedlove, Sex difference and laterality in the volume of mouse dentate gyrus granule cell layer, Brain Res. 827 (1999) 41–45.
[74] T.J. DeVoogd, D.J. Pyskaty, F. Nottebohm, Lateral asymmetries and testosterone-induced changes in the gross morphology of the hypoglossal nucleus in adult canaries, J. Comp. Neurol. 307 (1991) 65–76.
[75] W.M. Kelso, M.E. Nicholls, G.L. Warne, M. Zacharin, Cerebral lateralization and cognitive functioning in patients with congenital adrenal hyperplasia, Neuropsychology 14 (2000) 370–378.
[76] I.M. Schwarz, L.J. Rogers, Testosterone: a role in the development of brain asymmetry in the chick, Neurosci. Lett. 146 (1992) 167–170.
[77] G.C. Westergaard, T.J. Chavanne, I.D. Lussier, S.J. Suomi, J.D. Higley, Hormonal correlates of hand preference in free-ranging primates, Neuropsychopharmacology 23 (2000) 502–507.
[78] K.A. Pfannkuche, A. Bouma, T.G.G. Groothuis, Does testosterone affect lateralization of brain and behaviour? A meta-analysis in humans and other animal species, Philos. Trans. Roy. Soc. B. 364 (2009) 929–942.
[79] J.V. Zappia, L.J. Rogers, Sex differences and reversal of brain asymmetry by testosterone in chickens, Behav. Brain Res. 23 (1987) 261–267.
[80] G. Richards, T. Beking, B.P.C. Kreukels, R.H. Geuze, A.A. Beaton, T. Groothuis, An examination of the influence of prenatal sex hormones on handedness: Literature review and amniotic fluid data, Horm. Behav. 129 (2021) 104929.
[81] R.E. Willemsen, A. Hailey, Sexual dimorphism of body size and shell shape in European tortoises, J. Zool. 260 (2003) 353–365.
[82] S. Duro, O. Gündemir, B. Sönmez, T. Jashari, T. Szara, G. Pazvant, A. Kambo, A Different Perspective on Sex Dimorphism in the Adult Hermann's Tortoise: Geometric Morphometry, Zool. Stud. 60 (2021) e9.
[83] M. Santacà, A. Wilkinson, G. Stancher, V.A. Sovrano, A. Bisazza, Lizards and tortoises show evidence of low inhibitory control, Sci. Rep. 15 (2025) 1–13.