

The effect of age, sex and gonadectomy on dogs' use of spatial navigation strategies

Anna [Scandurra](#)^{a, b}

Lieta [Marinelli](#)^{a, *}

lieta.marinelli@unipd.it

Miina [Lööke](#)^c

Biagio [D'Aniello](#)^b

Paolo [Mongillo](#)^a

^aLaboratory of Applied Ethology, Department of Comparative Biomedicine and Food Science, University of Padua, Viale dell'Università 16, 35020, Legnaro (PD), Italy

^bDepartment of Biology, University of Naples "Federico II", Via Cinthia, 80126, Naples, Italy

^cInstitute of Ecology and Earth Sciences, University of Tartu, Vanemuise 46, 51014, Tartu, Estonia

*Corresponding author.

Abstract

In this study we assessed the effect of sex and gonadectomy on the type of spatial strategy (allocentric or egocentric) preferentially used by dogs in the acquisition of a navigation task and their ability to resort to the non-preferred strategy. Fifty-six dogs were involved in the study, divided in four equally sized groups based on sex and gonadectomy. Dogs initially underwent a learning phase, where they entered a plus-shaped maze from one arm and had to learn the position of a food bowl, which was placed in one of the lateral arms. The task could be achieved by relying on an either egocentric (i.e. learning to turn left or right) or allocentric strategy (i.e. using the external cues provided within the maze as a reference the position of the baited bowl). Following training, dogs were let in the maze from the entrance opposite to the one used in the learning phase, so that use of an egocentric strategy would lead them to search for food in one arm, while using an allocentric strategy would lead them into the opposite arm. Dogs' choices were used to determine their preferred strategy. In the last training phase, we assessed dogs' ability to resort to their non-preferred strategy to find the baited food bowl, by removing external cues and placing the baited bowl always at the same side of the dog, for subjects deemed as allocentric, and by keeping external cues and placing the baited bowl in a constant location relative to the cues, for dogs deemed as egocentric. No effect of sex was found on strategy preference, but ovariectomized females were significantly more likely to prefer an egocentric strategy, implying a role of ovarian hormones in biasing navigation strategies. The probability of resorting to the non-preferred strategy increased with aging in females and decreased in males. The higher requirement to cope with unpredictable environments during dispersal may support a predisposition to flexibly use different sources of information in younger males. By contrast, experience may be needed by females to reach the same proficiency, thereby justifying the increase in flexibility with ageing. In addition to increasing our knowledge about navigation, these results highlight effects of sex and ovariectomy on dog cognition, with potentially important implications regarding the management of dogs in different fields.

Keywords: Dog; Gonadectomy; Plus-maze; Sex; Spatial cognition; Strategy

1 Introduction

Spatial navigation entails cognitive processes that allow mobile animals to know where they are and to find a way back to their shelters, or to access resources, by using multiple cue sources, such as path integration, magnetic cues and different landmarks ([Brodbeck and Tanninen, 2012](#)). The spatial cognitive processing requires memorizing specific landmarks, positions and locations, allowing, in its most sophisticated form, to elaborate a cognitive map in order to orientate oneself and navigate in the surrounding environment.

In the last decades, a body of researches underlined sex differences in spatial navigation tasks in mammals with males showing generally better performances, possibly due to a different involvement in the reproductive function ([Astur et al., 2004](#); [Clint et al., 2012](#); [Hawley et al., 2012](#); [Healy et al., 2009](#); [Shah et al., 2013](#)). On the one hand, males' advantage in solving spatial navigation tasks could have been inherited from competition for mating, that

in most cases encompasses an extended home range (Ecuyer-Dab and Robert, 2004; Macdonald and Carr, 1995; Pal et al., 1998) compared to females. Such sex differences are typical of polygynous, rather than monogamous species, underlining the link between reproductive strategies and spatial cognitive abilities (Ecuyer-Dab and Robert, 2004). On the other hand, females' higher involvement in protection of the offspring, may have favoured a superior spatial ability in spatially restricted areas, using memory of nearby spatial information (Ecuyer-Dab and Robert, 2004; Guillamón et al., 1986; Herman and Wallen, 2007). Different navigation strategies have also been highlighted, with males seemingly preferring an allocentric navigation - i.e. using the relative position of the objects inside the surrounding space to orientate - and females exhibiting a bias for egocentric navigation strategy, referring prevalently to their motor responses (Hawley et al., 2012; Herman and Wallen, 2007; Jonasson et al., 2004).

Sexual hormones are one of the physiological factors driving these sex differences in spatial navigation. Sexual hormones act at organizational level, shaping the brain during development (Isgor and Sengelaub, 1998; Williams et al., 1990), but they also have activational effects, leading to differences between sexes (or even within one sex, e.g. across the oestrous cycle) in adult individuals (Daniel, 2006; Martin et al., 2007). For instance, a detrimental effect of ovariectomy has been observed in the acquisition of radial arm maze (Daniel et al., 1999). Furthermore, castration adversely affects working memory (a limited capacity resource used for temporarily preserving information while simultaneously processing the same or other information) during navigation, but it does not significantly impair reference memory (a long-lasting memory used to store information that remains constant over time) in male rats (Gibbs and Johnson, 2008; Locklear and Kritzer, 2014). Ovariectomized female rats show worse navigation abilities, which encompass a decrease in both working and reference memories (Gibbs and Johnson, 2008), whereas oestrogen administration quickly improves spatial reference memory in reproductively quiescent female mice (Frick et al., 2002).

Dogs, as polygynous species with a different involvement in reproduction between sexes, are a good candidate to study sex differences in spatial navigation. Dogs showed a great ability to solve different spatial tasks, thanks to the use of a wide range of spatial skills. They have better memory for spatial locations presented before in a spatial list (primacy effect) rather than ones presented later (recency effect) (Craig et al., 2012). They are capable of integrating spatial signals during locomotion, continuously updating the information about the distance and direction from an object (path integration; Cattet and Etienne, 2004) and developing novel paths based on knowledge of paths already used before (Séguinot et al., 1998). In a landmark-based search paradigm, dogs proved to be able to encode spatial information related to local and global allocentric cues (Fiset 2009, 2007). They can use both egocentric and allocentric references in different type of tasks (Cattet and Etienne, 2004; Chapuis et al., 1983), showing to prefer egocentric strategy to solve an object finding task and to flexible switch to the non-preferred strategy when the preferred one became useless (Fiset et al., 2000; Fiset and Malenfant, 2013). In a social learning paradigm, we recently showed that dogs preferentially relied on allocentric information in matching the location of the owner's demonstration (Fugazza et al., 2017). On the other hand, dogs were able to learn an egocentric strategy when allocentric cues were made unreliable, with males more skilful than females.

Maze paradigms represent a valid tool in canine models to study the functions involved in spatial navigation (Craig et al., 2012; Macpherson and Roberts, 2010; Mongillo et al., 2015, 2013; Parson et al., 2016) and the plus-maze has been proved one of the most effective type to assess navigation strategies both in humans and laboratory animals (Harris et al., 2012; Packard, 2009). A previous study performed by our research group demonstrated the feasibility of a T-maze paradigm to study spatial learning, long term memory and reversal learning (i.e. flexibility), showing an impairment in reversal learning abilities in older dogs (Mongillo et al., 2013). We also showed that female dogs were faster than males in acquiring a spatial learning task, but no difference emerged between sexes in the reversal learning task (Mongillo et al., 2017). However, gonadectomy affected spatial learning in females, with ovariectomized females performing significantly worse than intact subjects, whereas no effect was found for the orchietomy in male dogs (Mongillo et al., 2017). While these studies underline the importance of sexual hormones in driving spatial cognitive bias, they also point out the importance of studies aimed to disentangle the effect of gonadectomy on cognitive skills. One of many questions to be answered is how sex and gonadectomy affect spatial navigation and memory in dogs. The inclusion of gonadectomized subjects, will help towards a better understanding of the mechanisms underpinning sex differences at functional level, and shedding more light on the effect of this common surgical practice on the dog's cognitive skills.

To the best of our knowledge, there are no studies investigating the effect of the gonadectomy in the use of egocentric or allocentric strategies during spatial navigation in dogs. Thus, the aim of this research was projected to evaluate the effect of the sex and gonadectomy on the type of strategy preferentially used by dogs in the resolution of spatial navigation tasks. Specifically, we first assessed whether dogs would preferentially use allocentric or egocentric reference frame in acquiring the plus maze paradigm. Subsequently, we assessed dogs' ability to resort to their non-preferred strategy, when such strategy becomes inadequate to solve the task. In view of previous literature pointing to a prominent role of ovarian hormones in influencing spatial cognition, we expect to find the largest differences between intact and ovariectomized females in the use of spatial strategies. In addition, based on our own findings - although produced in substantially different tasks - we may expect females to outperform males in the initial acquisition tasks, but males to outperform females in the acquisition of the non-preferred strategy task.

2 Materials and methods

2.1 Subjects

Fifty-six healthy pet dogs were recruited through advertisements in veterinary clinics, parks, and the University of Padua. Recruitment criteria included age between two and eight years, and high motivation for food, which was assessed by the experimenter by presenting the dogs a piece of sausage, just prior to the beginning of the experimental procedures. In addition, sex and gonadectomy were taken into account for recruitment so that four groups of

the same size ($N = 14$) were formed, including intact (non-orchietomized) males (IM, mean age \pm SD: 4.4 ± 2.4 years), orchietomized males (OM, 4.4 ± 1.2 years, orchietomized at 18.0 ± 14.2 months of age), intact (non-ovariectomized) females (IF, 3.4 ± 1.5 years) and ovariectomized females (OF, 4.0 ± 1.9 years, ovariectomized at 17.2 ± 12.8 months of age) of different breeds were formed.

Experimental groups did not significantly differ for the mean age ($F = 0.94$; $P = 0.43$); there was no difference in age at gonadectomy between gonadectomized males and females ($t = 0.15$, $P = 0.88$).

All tests were conducted at the Laboratory of Applied Ethology (Department of Comparative Biomedicine and Food Science, University of Padua) in a room of about 5×5 m.

2.2 Assessment of preferred strategy

This procedure was meant to determine for each dog the preferred strategy in the acquisition of a simple navigation task. The procedure included a learning phase, where dogs had to learn the position of a food bowl within a plus maze, followed by a strategy assessment trial, where egocentric and allocentric frames of reference were put in contrast, allowing to point out each dog preferred strategy. The learning phase and the subsequent assessment test were administered twice or three times as detailed below, to determine the consistency of dogs' preferred strategy and classify dogs as preferentially 'egocentric' or 'allocentric'.

2.2.1 Experimental setting

In this part of the procedure, we used a plus-shaped maze (Fig. 1). The maze was made of plastic panels with a height of 2.0 m. Two of the maze arms (the North arm, N, and the South arm, S) were 1.5 m long and had a door at their end. The two lateral arms (East, E, and West, W), were 1.0 m long and were closed by a fixed panel. The four arms were 0.80 m wide, so the squared central area of intersection between the four arms had a dimension of 0.80×0.80 m. The floor of the maze was covered with grey PVC. Cues were placed inside the maze as spatial references: a black stake was placed approximately 0.50 m from the S door, and alternated strips of black and grey PVC were arranged in the shape of a triangle on the floor across the long arms, with the base close to the S door, and the apex close to the N door. Two empty food bowls were placed near the end of both the E and W arms. Outside the maze, four large white tents, covered the walls of the room to prevent the use of objects (e.g. windows, radiator) as landmarks.

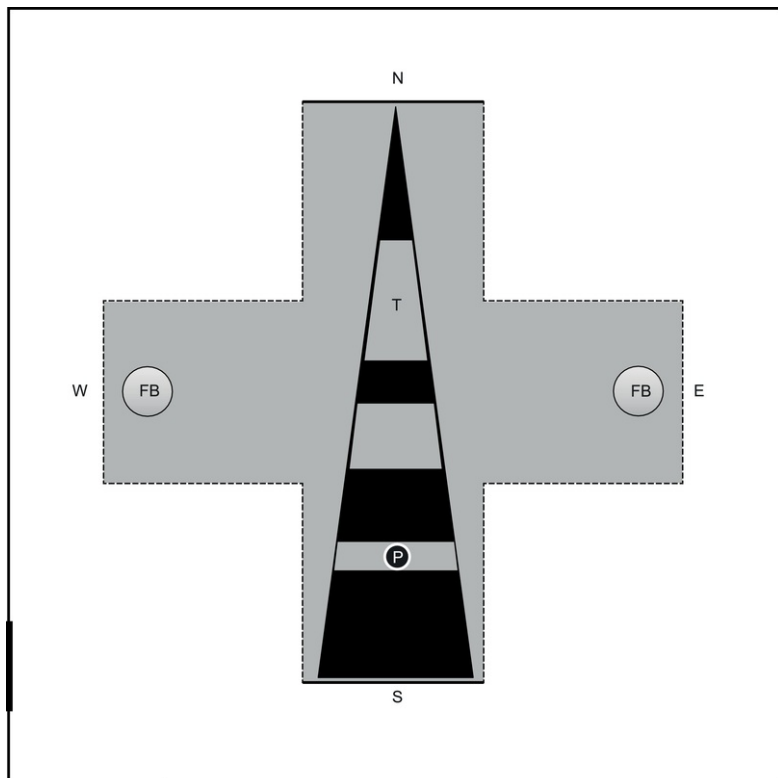


Fig. 1 Schematic representation of the plus-maze. The south (S) and north (N) arms' doors (continuous lines) were used as entrance point in the Learning task and Strategy assessment trials, respectively; the east (E) and west (W) arms were used as location for the food bowls (FB). Grey and black pieces of PVC mat, arranged in a triangular shape, (T) and a pole (P) placed in the S arm represented landmarks to be (potentially) used as allocentric cues.

alt-text: Fig. 1

2.2.2 Preliminary phase

This phase lasted about 10 minutes and was meant to familiarize the dog with the room and the maze. Owners were invited to enter the test room with their dogs kept on lead and to walk around the maze. After a full round was completed, the S and N doors were opened, and the owner were asked to walk inside the maze from door S to N, get out of the maze, turn back and, repeat the path backwards, without allowing dogs to enter the short lateral arms of the maze. The experimenter closed the doors S and N and then briefly explained the next steps of the procedure.

A side choice trial was performed to assess the dogs' first choice for turning into the E or W arm. Door S was opened, and the dog was released by the owner inside the maze. As soon as the dog reached the empty food bowl at the end of either lateral arm, the owner reached the dog, attached the lead and got out of the maze, preventing the dog from exploring the opposite short arm. The arm not visited by the dog was then identified as the 'correct' arm (where the baited food bowl was placed) for the following learning phase.

2.2.3 Learning task

In this task, dogs had to learn, across consecutive trials, in which of the lateral arms they could find a bowl with food. Prior to each trial, the experimenter put some food (few pieces of sausage) in the bowl in the correct arm; the same amount of food was placed under the non-baited bowl, not reachable by the dog to balance for odour cues. Door S was opened, and the owner and dog stood at the maze's entrance for a five second interval, which was intended to allow the dog to inspect the inside of the maze, while the owner was instructed to look down and remain as neutral as possible. After such interval, the dog was released, while the owner remained at the maze entrance. When the dog reached one of the bowls at the end of either arm E or W (as detected through the CCTV), the owner was told to enter the maze and quickly recover the dog, preventing it from visiting the opposite arm. The trial was considered correct if the dog entered the arm with the baited bowl, or incorrect otherwise. Trials were repeated until the dog made 5 correct choices in a row, in a maximum of 16 trials. If the criterion was not met within the 16th trial, the dog did not proceed to the next phases.

2.2.4 Preferred strategy assessment trial

When the dog reached the learning criterion, a strategy assessment trial was performed. Prior to the trial, the owners were instructed to walk around the maze once clockwise and then once counter-clockwise, with the aim reducing the possibility that dogs used external cues to orientate. The subsequent procedure for this trial was similar to that described for the trials of the learning task, with the exception that food was placed and accessible in the bowls at the end of both arms E and W, and dogs entered the maze from door N, opposite to the one used in the previous learning trials. The rationale for this trial was that allocentric and egocentric frames of reference were now contraposed and reliance on one or the other would result in the choice of a different arm: if the dogs based their choice on the spatial relationship between the cues present in the maze, they would go into the same arm as previously learned and their strategy in this trial would be considered 'allocentric'; if they relied on a motor response, that is turning towards their right or left as learned in the previous learning phase, they would go into the opposite arm as previously learned and their strategy would be considered 'egocentric'.

2.2.5 Assessment of consistency in preferred strategy

The learning task and the strategy assessment trial were repeated, with the same procedure and criteria described above. If the preference in such second assessment trial was consistent with the first assessment, the dog was classified as preferentially 'allocentric' or 'egocentric', according to their choice and proceeded to non-preferred strategy phase. If the two assessment trials were incoherent, a third learning task was performed followed by another assessment trial, which was used to classify dogs as allocentric or egocentric.

2.3 Use of non-preferred strategy

The aim of this phase was to assess the dog's ability to learn to use navigation strategies in a flexible way, that is to resort to the non-preferred strategy, when the preferred one is no longer usable to solve the task. This phase began after an interval of 15 minutes from the previous phase. The experimental setting was changed according to the type of strategy preferred by dogs, resulting from the strategy assessment, and will be described below in detail. In either case, the phase involved the administration of several trials, until dogs reached a learning criterion of five consecutive correct responses, within a maximum of 32 trials. A 10-minute break was allowed to dogs after the 16th trial and after the 24th trial. The procedure was terminated when the dog reached the learning criterion, or after the dog completed the 32nd trial.

This phase was preceded by a single trial to confirm that the new setting has not interfered with learning and the dog prefers the bowl chosen in the learning task with its preferred strategy.

2.3.1 Forced allocentric learning task

This procedure was administered to dogs that had shown a preference for the egocentric strategy in the previous phase. As shown in Fig. 2, the maze's walls were removed, avoiding that dogs were forced to make an obvious body turn (when turning from the entrance arm towards the lateral arm), to reach the food bowl. The PVC triangular floor, the stake and the bowls were maintained in the same relative positions (e.g. the bowl containing food always to the right side of the triangle seen from its base; the empty bowl to its left). To bias the dog toward an allocentric strategy, the starting point was alternated every other trial between the base and the tip of the PVC triangle, so that the dog had to rely on the relative position of the spatial cues to find the correct bowl, rather than turning to its right or left. After five seconds that the dyad was at the starting position, the dog was released. As soon as the dog had chosen one of the two bowls, the owner reached the dog, attached the lead and left the room for few seconds, allowing the experimenter time to bait the bowl for the next trial. In order to avoid the possibility that dogs remembered a specific motor sequence across trials when entering the room, the dog and owner used a different door at each trial to leave/enter the room. Furthermore, the dyad moved in the room behind the curtains to reach the starting position in each trial, so that the dog could not approach the bowls or other items before arriving at the starting point.

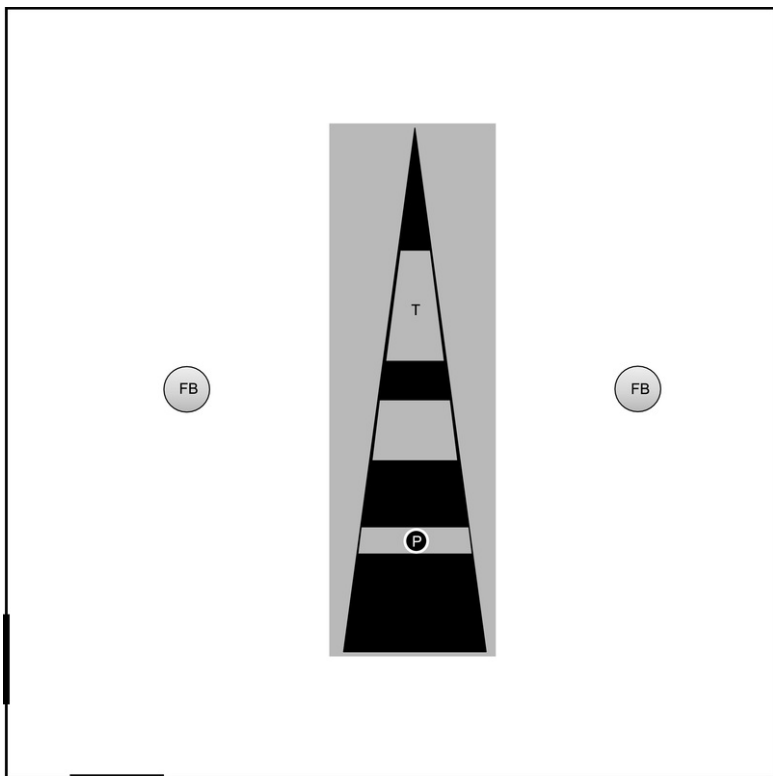


Fig. 2 Schematic representation of the experimental setting of the Forced allocentric task. A stake (St) and grey and black pieces of PVC mat arranged in a triangular shape (T) represented landmarks to be used as allocentric cues. Food bowls (FB) were always placed at the side of the triangle. A and B: doors alternatingly used by the owner and dog to leave/enter the room between trials.

alt-text: Fig. 2

2.3.2 Forced egocentric learning task

This procedure was administered to dogs that had shown a preference for the allocentric strategy in the previous phase. To prevent dogs from relying on the landmarks used in the previous phases, the PVC triangle and the stake were removed and the whole plus-maze was rotated by 45° (Fig. 3). To induce dog to use an egocentric strategy, four different starting point were randomly alternated across trials (S, N, E and W) and, in each trial, the baited bowl was always placed in the arm on the same side (either left or right) of the one used for entrance.

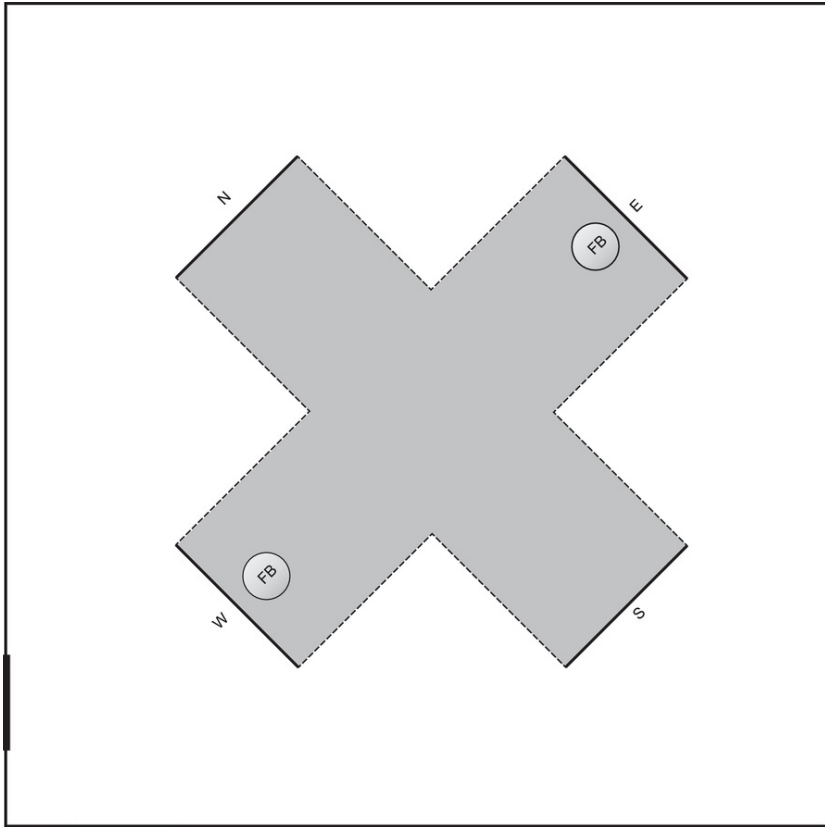


Fig. 3 Schematic representation of the experimental setting in the Forced egocentric task. The south (S), north (N), east (E) and west (W) arm were all alternatingly used as starting point and food bowl location in different trials of this phase.

alt-text: Fig. 3

2.4 Data collection and analysis

All tests were video-recorded and data of choice were obtained by coding the videos with the Observer XT Ver. 12.5 software (Noldus, The Netherlands). Dogs' responses (correct/incorrect) were collected in the Learning task of the Assessment of preferred strategy phase and in the Forced strategy tasks of the Use of non-preferred strategy phase. This data was used to calculate the number of errors made and the number of trials needed to reach the learning criterion in each of these tasks. The strategy (i.e. egocentric or allocentric) adopted by dogs in each of the Strategy assessment trials was recorded, and two trials with consistent strategy were used to classify dogs as either preferentially allocentric or egocentric. Trial outcomes were collected from all videos by a second observer, which resulted in a 100% agreement between the two observers.

The data from the preferred strategy assessment phase was analysed to determine if there was a bias – in the overall sample, or in relation to sex and gonadectomy – for either the allocentric or the egocentric strategy, and if the preferred strategy was associated with a difference in the ease of acquisition of the learning task.

To determine if there was a bias for either strategy in our sample, a binomial test was run using as a dependent variable the dog classification as preferentially egocentric or allocentric; the test was performed both the whole sample and within each of the four experimental groups (IM, OM, IF, and OF). If a strategy bias emerged in any of the groups, a subsequent Fisher exact test was run to determine whether the distribution of preferred strategy differed between dogs of different sex and/or gonadectomy status.

To determine if the preferred strategy was associated with a different ease of acquisition of the Learning task, univariate ANOVAs were used. Separate tests were run for the number of trials needed to reach the learning criterion and the number of errors made on the first Learning task of the Preferred strategy assessment phase. Independent variables were the dog's sex, gonadectomy status within sex, age, and preferred strategy. The analysis was

restricted to the first learning task, since it was the only one that required learning something novel, whereas the second, and the eventual third learning tasks, necessarily had a reduced learning requirement.

Data from the Use of non-preferred strategy phase (pooling data from the Forced allocentric and Forced egocentric tasks) were analysed to determine whether sex, gonadectomy, age and the type of preferred strategy affected the probability and the ease with which dogs resorted to their non-preferred strategy.

To determine the role of factors associated with successful outcome of the forced strategy task, a generalized linear model was used, with a binary link function. The model included the outcome of the Forced strategy task as a binary dependent variable; the dog's sex, gonadectomy nested within sex, and preferred strategy were fitted in the model as two-level fixed factors, and the dog's age as a covariate. The model also included first- and second-order interactions between all factors and the covariate. The final model was obtained by a backwards elimination, starting from higher order and non-significant interactions, and comparing fitting parameters, to identify the model with the best fit.

To determine if the dog's sex, gonadectomy, age, or preferred strategy were associated with a difference in the ease of acquisition of the Forced strategy task, univariate ANOVAs were used. Separate tests were run for the number of trials needed to reach the learning criterion and the number of errors made. Independent variables were the dog's sex, gonadectomy within sex, age, and preferred strategy.

All analyses were performed with SPSS software (SPSS ver. 24, IBM, Armonk, NY, USA). Data are reported as mean \pm SD unless otherwise stated.

3 Results

3.1 Assessment of preferred strategy

All the 56 dogs acquired the Learning tasks of the Strategy assessment phase.

Forty-three dogs (76.8% of the sample) choose a consistent strategy in the first and second assessment trial. Details of the strategies preferred by dogs and the number of assessment trials required to determine their preferred strategy are reported in Table 1. With 60.7% dogs using an egocentric strategy and 39.3% an allocentric one, there was no overall bias towards the use of either strategy ($P = 0.14$). No strategy bias was found within intact males ($P = 0.79$), orchiectomized males ($P = 1.00$) and intact females ($P = 1.00$); however, ovariectomized females were significantly more likely to prefer an egocentric strategy ($P = 0.01$) revealing an association between gonadectomy and strategy preference in females (one-sided Fisher's Exact Test, $P = 0.05$).

Table 1 Number of dogs of each experimental group preferring an egocentric or an allocentric strategy in the Preferred strategy assessment phase; the number within parenthesis indicates the number of dogs needing three repetitions for the determination of the strategy preference.

alt-text: Table 1

| Sex group | Preferred strategy | |
|-----------------------|--------------------|-------------|
| | Egocentric | Allocentric |
| Intact male | 8 (1) | 6 (1) |
| Orchiectomized male | 7 (2) | 7 (1) |
| Intact female | 7 (2) | 7 (1) |
| Ovariectomized female | 12 (3) | 2 (2) |
| Total | 34 (8) | 22 (5) |

Dogs needed an average of 10.0 ± 4.8 trials to reach the learning criterion in the first learning task, 6.7 ± 2.6 in the second and 7.2 ± 2.9 in the third. The average number of errors made was 2.4 ± 2.6 in the first Learning task, 0.7 ± 1.0 in the second and 0.9 ± 1.5 in the third. No effect was found for the dogs' sex, gonadectomy within sex, age, or preferred strategy on trials to criterion or errors of the first Learning task (Table 1, Table 2).

3.2 Use of the non-preferred strategy

Overall, the *Forced strategy task* was successfully completed by 66.1% ($N = 37$) of the dogs. An interactive effect of sex and age was found on the outcome of this phase ($\chi^2 = 4.04$, $P = 0.04$), with the probability of succeeding increasing in females and decreasing in males, with increasing age (Fig. 4). No effect of gonadectomy within sex, preferred strategy, or age at gonadectomy was observed on dogs' likeliness to succeed in this phase (gonadectomy:

$\chi^2 = 0.02$, $P = 0.99$; preferred strategy: $\chi^2 = 0.00$, $P = 0.96$; age at gonadectomy: $\chi^2 = 1.40$, $P = 0.24$ (Table 2).

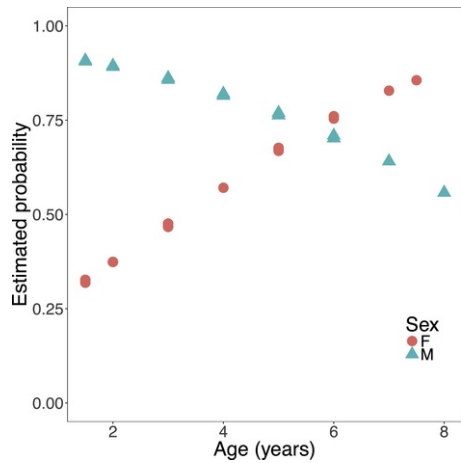


Fig. 4 Estimated probability to succeed in the Forced strategy task by male and female dogs of different age ($\chi^2 = 4.04$, $P = 0.04$).

alt-text: Fig. 4

Table 2 Effect of sex, gonadectomy within sex, age and preferred strategy as resulting from the first Preferred strategy assessment trial, on the number of trials needed by dogs to reach the learning criterion, the number of errors made and the average trial latency in the first Learning task. Univariate ANOVA.

alt-text: Table 2

| Variable | Factor | DF | F | P |
|---------------------|--------------------|----|------|------|
| Trials to criterion | Sex | 1 | 0.89 | 0.35 |
| | Gonadectomy (Sex) | 2 | 0.29 | 0.75 |
| | Age | 1 | 0.37 | 0.55 |
| | Preferred strategy | 1 | 0.59 | 0.44 |
| Errors | Sex | 1 | 2.96 | 0.09 |
| | Gonadectomy (Sex) | 2 | 0.46 | 0.63 |
| | Age | 1 | 0.20 | 0.65 |
| | Preferred strategy | 1 | 0.01 | 0.91 |

The average number of trials needed to reach the learning criterion was 16.30 ± 8.40 , with 5.76 ± 4.63 errors. As reported in Table 3, no effect of sex, gonadectomy within sex, age, or preferred strategy was found on the number of trials needed to reach the learning criterion, the number of errors made in the Forced strategy task.

Table 3 Effect of sex, gonadectomy within sex, age, age at gonadectomy and preferred strategy as resulting from the Preferred strategy assessment phase, on the number of trials needed by dogs to reach the learning criterion, the number of errors made and the average trial latency in the Forced strategy task. Univariate ANOVA.

alt-text: Table 3

| Variable | Factor | DF | F | P |
|---------------------|--------|----|------|------|
| Trials to criterion | Sex | 1 | 0.64 | 0.43 |

| | | | | |
|--------|--------------------|---|------|------|
| | Gonadectomy (Sex) | 2 | 1.41 | 0.26 |
| | Age | 1 | 0.08 | 0.78 |
| | Preferred strategy | 1 | 0.09 | 0.77 |
| Errors | Sex | 1 | 0.09 | 0.77 |
| | Gonadectomy (Sex) | 2 | 0.87 | 0.43 |
| | Age | 1 | 0.59 | 0.81 |
| | Preferred strategy | 1 | 0.02 | 0.88 |

4 Discussion

In this study, we observed no overall prevalence in dogs' choice of an egocentric (response) or allocentric (place) strategy to acquire a simple spatial task. This was not an unexpected result, as the maze and the procedure were deliberately devised to be easily solvable with both strategies and dogs have proven able to use different sources of information in solving spatial tasks (Chapuis et al., 1983; Fiset et al., 2000; Fiset and Malenfant, 2013). However, ovariectomized females showed a clear preference for using an egocentric strategy, indicating a potential role for ovarian hormones in strategy use. Ovarian hormones have a complex effect on different cognitive domains implied in the acquisition of spatial tasks. For instance, ovariectomy decreases working and reference memories in rat (Gibbs and Johnson, 2008), but oestrogen administration improves spatial reference memory in mice (Frick et al., 2002). As regards navigation strategies, most studies seem to converge on the fact that ovarian hormones determine a bias towards the use of allocentric rather than egocentric strategies. For instance, the acute administration of oestrogen to ovariectomized rats enhances performance in place-learning tasks and reduces performance in response-learning based tasks (Gold, 2001; Korol and Manning, 2001; Korol and Kolo, 2002). In addition, when free to choose the preferred strategy, female rats prefer allocentric strategies during pro-oestrous, when circulating levels of ovarian hormones peak (Korol et al., 2004). Our results are in agreement with such findings, indicating that also in the dog the absence of ovarian hormones results in a bias towards egocentric strategies to solve a spatial task.

An effect of ovariectomy on dog's performance in a spatial task has earlier been observed in our lab, where an advantage of non-ovariectomized females in the acquisition of a spatial task was found (Mongillo et al., 2017). As no allocentric cues were provided in such task, the better performance of intact females was tentatively explained by hypothesizing that a prevalence existed in this population for the use of an egocentric strategy. It therefore remains to be explained why we did not see either an advantage in terms of learning speed, nor a prevalence in egocentric strategies by intact females in the present experiment. One possibility is that the effects of ovariectomy only emerge when one strategy is particularly advantageous. In other words, intact females may easily resort to egocentric strategies when this is the only or the most obvious way to solve a task, but lose such preference, and consequently the advantage reported in Mongillo et al. (2017), when allowed to freely choose the strategy, as was the case of the present procedure. Supporting these views, administration of ovarian hormones in ovariectomized rats facilitates the use of egocentric strategies only when this represents the most efficient way to solve a task (Bimonte-Nelson et al., 2006).

In addition to the availability of different strategies, other methodological aspects may help explain the different effect of ovariectomy on dogs' performances between the two studies. For instance, the maze of the present study was simpler in its navigation, and its entire inner development was clearly visible from the centre of the maze, thus a choice could be made when the final destination was in sight. On the contrary, in the previous study the end of the arms was not visible, implying a more complex mapping/mnemonic effort by dogs. These differences may have resulted in a general easier acquisition of the present task, and therefore its lower sensitivity in terms of learning speed. This is supported by the higher success rate (100% vs. 87.5%) and the lower number of errors made (2.4 ± 2.6 vs. 4.4 ± 3.8) in spite of a stricter learning criterion of the present task. In addition, in the task described in Mongillo et al. (2017) dogs were separated from the owner and learning how to navigate the maze was aimed at reuniting with him/her. Such situation could have represented a source of possible distress, which, in turn, may have impacted differently on the performance of intact and ovariectomized females, as suggested in other species and other cognitive domains (Andreano and Cahill, 2009).

The second part of the experiment showed that the probability of resorting to the non-preferred strategy increased in females and decreased in males as a function of increasing age. It is not easy to contextualize these results in the existing literature, since there is no unitary vision about age and sex differences in cognitive flexibility. One possibility is that the highest odds of resorting to a non-preferred spatial strategy observed in our younger males reflects a predisposition to flexibly use different sources of information. In most mammals, males tend to disperse more than females from their natal group (Greenwood, 1980), and, in dogs, the incidence of dispersal is higher in juveniles than in adults (Pal et al., 1998). Thus, our results could reflect the higher requirement to cope with unpredictable environments in juvenile male dogs during dispersal.

From a neurobiological perspective, this predisposition may find its roots in the differential activation of brain areas implied in spatial processing, in the course of brain maturation, as found between men and women (Rubia et al., 2010).

The improvement in performance observed in our females across the life span could reflect a role of experience, which females may need in order to learn to resort to different sources of spatial information. [Lacreuse and collaborators \(2005\)](#) showed that training completely reverses female disadvantage in solving a spatial task in young macaques. In addition, sex specific effects of training on spatial tasks have been reported in rats ([Perrot-Sinal et al., 1996](#)) and humans ([Goldstein and Chance, 1965](#); [Johnson et al., 1979](#); [Saccuzzo et al., 1996](#); [Stericker and LeVesconte, 1982](#); [Vasta et al., 1996](#)). Since our males were already close to the maximum possible performance at a young age, no further improvement could be expected during ageing. Nor, however, did we expect to find a decrement in males' flexibility. Some studies do report an association between age and poorer performance in tests of spatial ability in men ([Moffat et al., 2002](#)). Greater age-related decline in various spatial tasks in male than in females have also been reported in macaques ([Lacreuse et al., 2005](#)), and rats ([Lukoyanov et al. 1999](#); [Veng et al., 2003](#)). In dogs, however, deficits in spatial flexibility are found at an older age than that spanned by our sample (i.e. >8 years of age, [Mongillo et al., 2013](#)), and the risk of cognitive impairment is greater in female than in male dogs ([Azkona et al., 2009](#)). Based on these studies, and on the age of our subjects, we can exclude that the lower flexibility observed in our males represents a symptom of cognitive impairment. More likely, the trajectory observed in our males represents a physiological age-related decrement in cognitive flexibility, which is masked in females by their lower initial performance and the effects of experience.

5 Conclusion

The present study showed that dogs can use different strategies when acquiring a spatial navigation task. It showed that ovariectomy is associated with a preference for the use of egocentric information, and both sex and age impacting on the flexibility with which dogs can switch between the preferred and the non-preferred strategy. The results bear on different theoretical and applied aspects. First, they contribute to improve our knowledge about navigation strategies and the role of physiological factors implied in such abilities. In view of the variability of effects that hormones have in different taxa, our results could represent an important addition toward a comparative analysis and understanding of mechanism leading to efficient navigation. Second, our findings are one of few examples of non-reproductive effects of ovariectomy. Since ovariectomy is often suggested by veterinarians for dog population control and health prevention, an increased knowledge about its effects on behaviour and cognition is desirable in order to make more aware choices. Finally, the results are relevant to those dogs' population where spatial cognition is of particular importance, such as dogs used in working or leisure activities. For instance, ovariectomy of bitches might be a rather inconvenient choice in context where orientation rather than route learning is important (e.g. search and rescue dogs).

6 Ethical clearance

The study was conducted in accordance with all applicable international, national, and institutional guidelines for the care and use of animals.

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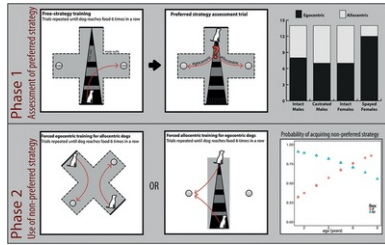
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Graphical abstract



Highlights

- Strategy preference in acquiring a spatial task was assessed in 56 pet dogs.
 - No overall preference emerged for egocentric or allocentric strategy.
 - Ovariectomized females preferred an egocentric strategy.
 - The ease of resorting to the non-preferred strategy was then assessed.
 - It increased with increasing age in females, and decreased with age in males.
-

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