

Christelle Alves · Raymond Chichery ·
Jean Geary Boal · Ludovic Dickel

Orientation in the cuttlefish *Sepia officinalis*: response versus place learning

Received: 29 June 2005 / Revised: 6 January 2006 / Accepted: 19 May 2006
© Springer-Verlag 2006

Abstract Several studies have demonstrated that mammals, birds and fish use comparable spatial learning strategies. Unfortunately, except in insects, few studies have investigated spatial learning mechanisms in invertebrates. Our study aimed to identify the strategies used by cuttlefish (*Sepia officinalis*) to solve a spatial task commonly used with vertebrates. A new spatial learning procedure using a T-maze was designed. In this maze, the cuttlefish learned how to enter a dark and sandy compartment. A preliminary test confirmed that individual cuttlefish showed an untrained side-turning preference (preference for turning right or left) in the T-maze. This preference could be reliably detected in a single probe trial. In the following two experiments, each individual was trained to enter the compartment opposite to its side-turning preference. In Experiment 1, distal visual cues were provided around the maze. In Experiment 2, the T-maze was surrounded by curtains and two proximal visual cues were provided above the apparatus. In both experiments, after acquisition, strategies used by cuttlefish to orient in the T-maze were tested by creating a conflict between the formerly rewarded algorithmic behaviour (turn, response learning) and the visual cues identifying the goal (place learning). Most cuttlefish relied on response learning in Experiment 1; the two strategies were used equally often in Experiment 2. In these experiments, the salience of cues provided during the experiment

determined whether cuttlefish used response or place learning to solve this spatial task. Our study demonstrates for the first time the presence of multiple spatial strategies in cuttlefish that appear to closely parallel those described in vertebrates.

Keywords Cuttlefish · T-maze · Side-turning preference · Place strategy · Response strategy

Introduction

According to Franz and Mallot (2000), navigation is the process of determining and maintaining a course or trajectory towards a goal location. This widespread ability is among the fundamental cognitive processes that animals require for survival. Spatial orientation allows animals to memorise and return to specific places, such as food patches, water sources and shelters or congeners.

Spatial learning systems have been extensively studied in both insects and vertebrates (Wehner et al. 1996; Healy 1998; Giurfa and Capaldi 1999; Golledge 1999; Dudchenko 2001). Given the differences in phylogeny and neural substrate underlying spatial memory, it appears that navigation processes have evolved independently in mammals, birds and hymenopterans (Benhamou and Poucet 1996). Despite the importance of studies in insects to our understanding this evolutionary convergence (Jacobs 2003), only a few studies have investigated spatial learning mechanisms in other invertebrate groups. Among cephalopods, spatially-related learning has been demonstrated in octopuses (Walker et al. 1970; Mather 1991; Boal et al. 2000) and in cuttlefishes (Karson 2003; Karson et al. 2003); however, the type of strategy and cues that are used to navigate remains to be determined. Cephalopods are often compared with vertebrates (fish and reptiles) because of the complexity of their nervous systems and their learning abilities (Packard 1972). Thus, studying navigation in this distinct taxon will provide important perspectives for better understanding the structure, function, and evolution of spatial learning and memory systems.

C. Alves · R. Chichery · L. Dickel (✉)
Laboratoire de Physiologie du Comportement des
Céphalopodes, Université de Caen,
Esplanade de la Paix,
14032 Caen cedex, France
e-mail: ludovic.dickel@unicaen.fr
Tel.: +33-2-31-56-55-83
Fax: +33-2-31-56-56-00

C. Alves · R. Chichery · L. Dickel
Centre de Recherches en Environnement Côtier,
54 rue du Dr. Charcot,
14530 Luc-sur-Mer, France

J. G. Boal
Department of Biology, Millersville University,
Millersville, PA 17551-0302, USA

113 Spatial learning can be achieved in several ways. Animals can solve a spatial task by learning an association between a response and a reward (response strategy) or they can learn an association between a given place and a reward (place strategy; Dudchenko 2001; Gibson and Shettleworth 2005). Previous studies have shown that response learning and place learning occur simultaneously in rodents (Gibson and Shettleworth 2005), but one of the two strategies can be favoured by the conditions of the experiment (Restle 1957). Previous studies have shown that fish employ multiple spatial strategies that closely parallel those described in mammals and birds (López et al. 1999). Although fish and cuttlefish have evolved independently, both had to cope with the same ecological constraints (Packard 1972). Comparable spatial memory processes could have emerged.

129 The aim of our study was to investigate whether cuttlefish learn a response and/or a place when they must return to a place previously visited, and whether experimental conditions can favour one of these two strategies. A new spatial learning procedure for cuttlefish was designed to answer these questions using a modification of a T-maze apparatus commonly used in vertebrates (rats, Restle 1957; Packard and McGaugh 1996; fish, Odling-Smee and Braithwaite 2003).

138 In rats, Andrade et al. (2001) demonstrated that initial preference for turning right or left (side-turning preference) in a T-maze influenced subsequent performance in a trained task. Subjects learn too easily when trained consistent with their side preference (ceiling effect) and learn only with difficulty when trained against their initial preference. A similar effect of initial preference on learning has been demonstrated in cephalopods (octopuses, reviewed in Boal 1996; cuttlefish, Karson et al. 2003). Byrne et al. (2002, 2004) suggested lateral asymmetry of eye use in *Octopus vulgaris*, and eye use patterns can affect maze performance in octopuses (Wells 1964). Our preliminary test aimed to confirm that cuttlefish show a spontaneous side-turning preference.

152 In Experiments 1 and 2, we asked whether cuttlefish would use a place or response-learning strategy to learn a simple orientation task, and whether the strategy preferentially used depended on the salience of the visual cues provided during the experiment. Preliminary observations of cuttlefish kept in laboratory tanks suggested that cuttlefish usually avoid open, lit areas when they cannot bury. In our experiments, the cuttlefish were rewarded for solving a T-maze task with time in a dark and sandy compartment at the end of one arm of the T-maze. Locating the dark compartment required the cuttlefish to either learn a body-centred algorithmic behaviour (turn left or right; response strategy) or orient using visual cues (place strategy). In Experiment 1, distal visual cues were provided around the maze. In Experiment 2, the T-maze was surrounded by curtains and proximal visual cues were provided inside this enclosure. To investigate which strategy they used to solve the maze problem, in each experiment, following training, cuttlefish received probe trials in which cues were manipulated.

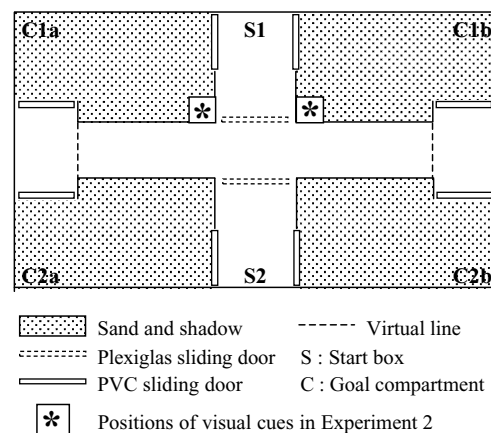
General method

Subjects

174 Sixteen adult cuttlefish (19–22 cm dorsal mantle length, approximately 1.5 years old) were trawled in January 2005 in the English Channel, and 16 additional subadult cuttlefish (15–20 cm dorsal mantle length, approximately 1 year old) were trawled in September 2005 in the vicinity of Luc-sur-mer (Calvados, France). Cuttlefish were housed individually in plastic tanks (80 cm × 60 cm × 40 cm) with circulating seawater at 15 ± 1°C. Cuttlefish were allowed to acclimate in the laboratory for 2 weeks before behavioural experiments began. They were fed either shrimp (*Cragon crangon*) or crabs (*Carcinus maenas*) once per day. Fifteen cuttlefish (six adults and eight subadults) were used in the preliminary test, 10 (adults) were used in Experiment 1, and seven other (subadults) were used in Experiment 2.

Apparatus

190 T-maze learning was assessed in a cross-shaped maze (see Fig. 1). The apparatus was constructed entirely from white PVC (200 cm long × 110 cm wide × 30 cm high) and was illuminated by a 300-W halogen lamp located 1 m above the centre of the maze. Two arms (40 cm long × 40 cm wide) were used as start boxes (S1 and S2; top and bottom arms of the cross-shaped maze in Fig. 1) while the other two arms (80 cm long × 30 cm wide) were used as goal arms and formed the maze alley (left and right arms of the maze in Fig. 1). At the end of each goal arm were two goal compartments (C; 80 cm long × 40 cm wide), one on the left and one on the right. The goal compartments were dark and covered with an opaque sliding PVC top. The bottom of each goal compartment was entirely covered with sand (1 cm deep). To form a T-maze, one start box and the immediately adjacent set of goal compartments were excluded by closing the three opaque sliding doors connecting them



171 **Fig. 1** Cross maze apparatus. The asterisk (*) indicates the position of the visual cues used in Experiment 2

to the maze alley. In this T-maze configuration, we used goal compartments C1a and C1b when the start box was S1, and C2a and C2b when the start box was S2. From the starting point (e.g. S1), the cuttlefish could not see if the entrance of the corresponding goal compartments were open or closed (e.g. C1a and C2a if the start box was S1). Sliding doors between goal compartments and start boxes permitted experimenters to move cuttlefish from a goal compartment to its corresponding start box with the minimum amount of handling. Seawater in the T-maze was 30 cm deep, with water flow provided between trials (preliminary test and the two experiments), to reduce water heating. Between sessions, water was totally renewed in the T-maze tank.

Statistical analysis

All data were analysed with non-parametric tests (Siegel and Castellan 1988) and computed using StatXact[®] and Systat[®] software. Criteria for side-turning preference and for acquisition were determined using a binomial test at a 5% level of significance and at 10% level of significance, respectively. Exact permutation tests for paired samples were used to compare performances between the first and the last session of acquisition and between the last session of acquisition and the probe sessions. Exact permutation tests for independent samples were used to compare the number of trials to acquisition between place- and response-learners. Exact chi-square test was used to compare the number of cuttlefish using each spatial strategy in Experiment 1. Fisher exact test was used to compare frequencies of the two strategies in the two experiments.

Preliminary test: side-turning preference test

The preliminary test aimed to confirm the possible existence of a spontaneous side-turning preference in cuttlefish that could influence learning performances in a T-maze.

Procedure

During this test, 15 cuttlefish (six adults and nine subadults) were tested in the T-maze configuration of the cross-shaped maze. One of the start boxes was blocked off and all the four goal compartments were closed. Eight cuttlefish were tested from start box S1, while the other seven cuttlefish were tested from start box S2. Each cuttlefish was placed individually in the start box for 15 s before the clear sliding door to the maze alley was removed. The cuttlefish was allowed to move freely out of the start box and into either arm of the maze. As soon as the cuttlefish chose an arm of the maze (movement of any part of the animal beyond the virtual line at the far end of an arm, see Fig.1), it was gently lifted out of water with a net and placed back into the start box. The arm-choice for each trial was recorded. If a cuttlefish did not turn into one of the two arms within

Table 1 Preliminary test: side-turning preference test

	Left-arm entry scores	Right-arm entry scores
Left preference ($n = 3$)	16 (0.88)	4 (0.88)
Right preference ($n = 8$)	4 (0.41)	16 (0.41)
No preference ($n = 4$)	9 (1.11)	11 (1.11)
Total sample ($n = 15$)	5 (1.35)	15 (1.35)

Cuttlefish have been characterised as ‘left preference’, ‘right preference’ (more than 15 trials out of 20, binomial test at the 5% level of significance) and ‘no preference’ animals

Data are medians (and SEMs) of the number of left- and right-arm entry scores for each group

10 min, the cuttlefish was removed and placed back into the start box. This procedure was repeated until 20 choices were made. Side-turning preference was determined using a criterion of 15 or more choices of the same arm (significant side-turning preference at 5% level of significance with a binomial test). In this way, cuttlefish were categorised into ‘left preference’, ‘right preference’ and ‘no preference’ (the remaining cuttlefish).

Results

Three of the 15 cuttlefish (20%) showed a significant left preference and eight (53%) showed a significant right preference (Table 1). Four (27%) cuttlefish did not show any preference. Of the 11 cuttlefish showing a significant preference, all preferred the side of their initial choice.

Discussion

In our study, 11 out of 15 (73%) cuttlefish showed a significant right- or left-turning bias in the T-maze. In this test, the first arm chosen by cuttlefish was always the statistically preferred side. Therefore, lifting animals out of the water did not seem to provide strong negative reinforcement.

Side-turning biases have influenced performances of rats in a T-maze (Andrade et al. 2001) and cuttlefish in an open-field maze (Karson et al. 2003). Consequently, in the subsequent experiments of our study, the side-turning preference of each cuttlefish was determined by the arm chosen during a first, unrewarded trial, and each cuttlefish was trained against its initial preference (away from its preferred maze arm).

T-maze spatial task: general training procedure

In the following two experiments, cuttlefish were tested individually in the T-maze configuration. For each cuttlefish, one of the start boxes was blocked off and the opposite start box was used for all training. We randomly assigned the start box (S1 or S2) used for each cuttlefish. Cuttlefish were given five trials per training session, with one training ses-

293 sion per day. For each cuttlefish, both goal compartments
294 were closed during the first trial of the first session to deter-
295 mine side-turning preference. During the remaining trials,
296 only the arm contralateral to their side-turning preference
297 was rewarded (goal compartment open).

298 During training trials, each cuttlefish was placed for 15 s
299 in the start box (“settling time”) before the clear sliding door
300 to the maze alley was removed. If the cuttlefish did not exit
301 the start box after 30 s (“start time”), it was gently chased
302 in the central alley using a small net. The settling time
303 and start time were chosen to allow the cuttlefish to calm
304 down in the apparatus, and are consistent with the previous
305 experiments (Karson et al. 2003). The cuttlefish was given
306 a maximum of 10 min to reach the end of one arm. Each
307 trial consisted of a unique choice run. Once the cuttlefish
308 reached and entered the goal compartment at the end of the
309 goal arm, the entrance to the goal compartment was blocked
310 off and the cuttlefish was allowed to remain in the dark, on
311 the sandy bottom, for 15 min. After 15 min had elapsed,
312 the sliding door separating the goal compartment from the
313 start box was removed and the cuttlefish was gently chased
314 into the start box using a small net. If the cuttlefish entered
315 the incorrect arm and consequently failed to reach the goal
316 compartment, the cuttlefish was immediately removed with
317 a net, transported through the air and placed back in the start
318 box. The criterion of error was the movement of any part
319 of the animal beyond the virtual line at the far end of the
320 incorrect arm (Fig. 1). Between trials, the water was stirred
321 to avoid the possibility that cuttlefish could use olfactory
322 cues to solve the maze problem.

323 Training continued until the cuttlefish reached a learning
324 criterion established as at least 80% correct responses over
325 two consecutive days of training (i.e. 8 correct choices out
326 of 10, significant choice at 10% level of significance with
327 a binomial test). Because of inter-individual differences,
328 such as mobility or stress, choice latency did not appear
329 to be a pertinent indicator. When a cuttlefish reached this
330 learning criterion, we conducted probe trials to determine
331 the strategy it used to solve the maze.

332 Experiment 1: T-maze spatial task with distal cues

333 In Experiment 1, ten adult cuttlefish were trained to solve
334 the spatial task in the T-maze (general training procedure).
335 In this experiment, cuttlefish could see out of the T-maze
336 and into the testing room, which contained various vi-
337 sual extramaze cues (water-pipes, sets of shelves. . .). The
338 light provided around the maze was homogeneous. Fol-
339 lowing training, two types of probe trials were conducted
340 to assess the type of orientation strategy the cuttlefish
341 used.

342 Start box reversal probe trials–response *versus* place
343 strategy

344 The day after reaching the learning criterion in the T-maze,
each cuttlefish received one probe session of five trials,

346 using the start box opposite the one used during training
347 (e.g. S2 if trained with S1). Both adjacent goal compart-
348 ments were left open (e.g. C2a and C2b if trained to C1a
349 and C1b) so that, whichever arm the cuttlefish chose, it
350 was rewarded with 15 min in a goal compartment. This
351 procedure prevented an ‘over-learning reversal effect,’ in
352 which animals given added trials show an increased ten-
353 dency to change behaviour when the reward contingencies
354 are changed (O’Keefe and Nadel 1978).

355 If the cuttlefish had simply learned a set of movements
356 (turn left or turn right, response strategy), then after re-
357 versing the starting position, it would make the same turn
358 (right if trained right, left if trained left) and travel down the
359 goal arm opposite the one travelled during training. Alter-
360 natively, if the cuttlefish had learned to orient with respect
361 to distal visual cues (place strategy), it would make the
362 opposite turn (left if trained right, right if trained left) and
363 travel down the same goal arm as the one travelled during
364 training.

365 If the cuttlefish chose the goal arm opposite the one re-
366 warded during training (at least four choices out of five;
367 same turn), it was categorized as a ‘response-learner’.
368 However, if the cuttlefish chose the same goal arm as
369 had been rewarded during training (at least four choices
370 out of five; opposite turn), a second probe trial was
371 conducted.

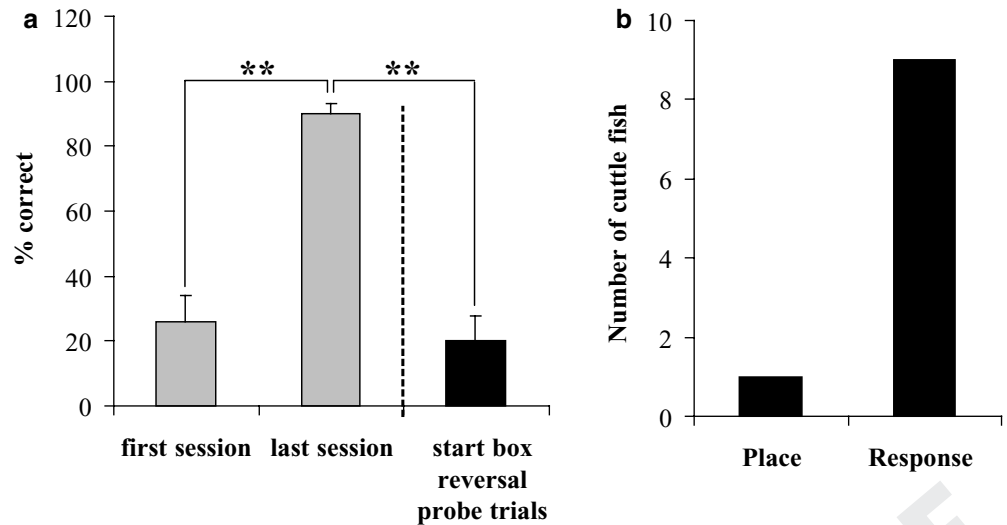
372 Curtained enclosure probe trials–intra-maze *versus*
373 extra-maze cues

374 The day after the first probe session, black curtains were
375 placed around the maze and yellow curtains were placed
376 above the maze. In this enclosure, each cuttlefish received
377 one session of five trials using the same start box as dur-
378 ing training, and with both adjacent goal compartments
379 open. In this probe session, if the cuttlefish no longer
380 consistently chose the goal arm rewarded during train-
381 ing, it would indicate that the cuttlefish had used extra-
382 maze cues to orient, and was categorized as a ‘place-
383 learner’. However, if the cuttlefish still consistently chose
384 the goal arm rewarded during training (at least four choices
385 out of five), it would indicate that the cuttlefish had
386 used visual cues within the maze imperceptible to the
387 experimenter.

388 Results

389 All cuttlefish reached the learning criterion in 3–10 ses-
390 sions (five trials per session), with a mean of 6 ± 1 sessions
391 (mean \pm SEM; i.e. 25–35 trials). The cuttlefish improved
392 their performances in reaching the goal compartment, as
393 indicated by the increase in correct choices. Percent suc-
394 cess (mean \pm SEM) was significantly higher during the
395 last session for each cuttlefish ($90 \pm 3\%$) than during its
396 first session ($26 \pm 8\%$; exact permutation test for paired
samples: $n = 10$, $P = 0.002$; see Fig. 2a).

Fig. 2 Experiment 1: **a** Mean percentages of correct choices per session (\pm SEM) during the first and last sessions of training and during the start box reversal probe trials. **b** Number of cuttlefish using the place or response arm during the start box reversal probe trials. The asterisks (**) indicates significant difference (exact permutation tests for paired samples: $P < 0.01$)



398 In the first set of probe trials (start box reversal), the mean
 399 number of correct choices (choice of the previously re-
 400 warding goal arm; 20 ± 8%) made by the cuttlefish was sig-
 401 nificantly lower than at the end of acquisition (90 ± 3%; ex-
 402 act permutation test for paired samples: $n = 10$, $P = 0.002$;
 403 see Fig. 2a). Analyses of individual performances showed
 404 that most cuttlefish (9 out of 10) used a response strat-
 405 egy, choosing the previously unrewarded goal arm at least
 406 four out of five times when the start box was inverted
 407 (exact chi-square test: response-learners: $n = 9$; place-
 408 learners: $n = 1$; $P = 0.011$; Fig. 2b). For the nine response-
 409 learner cuttlefish, the learning criterion during training was
 410 reached within 6 ± 1 sessions (mean ± SEM; i.e. 25–35
 411 trials).

412 One cuttlefish appeared to use a place strategy, consis-
 413 tently choosing the previously rewarded goal arm (five
 414 choices out of five). This cuttlefish had reached the learn-
 415 ing criterion during training after five sessions of tri-
 416 als (i.e. 25 trials). To test whether this cuttlefish relied
 417 on extramaze cues or on intra-maze visual cues imper-
 418 ceptible to the experimenter, we conducted the second
 419 probe trial (curtained enclosure). Blocking access to ex-
 420 tramaze cues resulted in a decrease in the consistency
 421 of goal arm choices for this cuttlefish from 100% to
 422 a chance performance of 40%. This cuttlefish appeared
 423 to be relying on distal visual cues external to the maze
 424 itself.

425 Discussion

426 Experiment 1 demonstrated that cuttlefish can solve this
 427 simple orientation task.

428 Results are consistent with those obtained with octopuses
 429 in a T-maze (Walker et al. 1970) and cuttlefish in a two-
 430 choice maze (Karson et al. 2003; Karson 2003); however,
 431 in the current experiment, subjects did not require any kind
 of shaping or pretraining.

In this experiment, a simple T-maze with distal visual
 cues, most cuttlefish (9 out of 10) relied on response learn-
 ing to orient.

Experiment 2: T-maze spatial task with proximal cues

In Experiment 2, seven cuttlefish were trained to solve
 the T-maze spatial task (general training procedure) when
 two salient cues were provided within a curtained area. To
 eliminate room cues, black curtains were placed around
 the maze and yellow curtains were placed above the maze.
 Two proximal visual cues were placed above the apparatus
 (5 cm above the water surface): a black-and-white striped
 PVC rectangle (15 cm high × 10 cm wide; stripes 3 cm
 wide) and a black-and-white spotted PVC square (15 cm
 wide; spots 3 cm in diameter; Fig. 1). Following training,
 probe trials were conducted to determine whether cuttlefish
 would preferentially use a place strategy when proximal
 visual cues were available.

Visual cue inversion probe trials – response *versus* place strategy

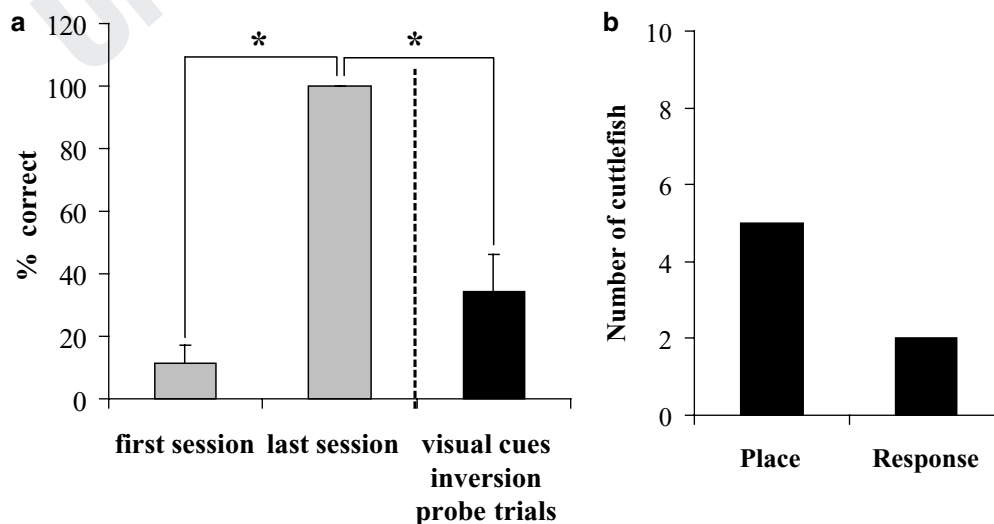
The day after the end of acquisition, the right/left positions
 of the two visual cues were reversed to create a conflict
 between the visual cues identifying the place and the right
 or left turn (response) learned during training. The cuttlefish
 received one session of five trials. If the cuttlefish still
 consistently choose the goal arm rewarded during training
 (at least four choices out of five for the previously re-
 warded goal arm; same turn), it would indicate that the cuttlefish
 had learned a response ('response-learners'). However, if
 the cuttlefish choose the opposite arm (at least four choices
 out of five for the previously unrewarded goal arm; opposite
 turn), it would indicate that the cuttlefish had learned to use
 visual cues to solve the maze ('place-learners').

466 All cuttlefish reached the learning criterion in 3–6 sessions (five trials per session), with a mean of 4 ± 1 sessions (mean \pm SEM; i.e. 15–25 trials). The cuttlefish improved their performances in reaching the goal compartment as indicated by the increase in the correct choice. 470 Percent success (mean \pm SEM) was significantly higher during the last session for each cuttlefish ($100 \pm 0\%$) 472 than during its first session ($11 \pm 6\%$; exact permutation test for paired samples: $n = 7$, $P = 0.016$; see Fig. 3a). 474 Mean percent success during the last session of acquisition was significantly higher in this experiment compared to the first experiment (exact permutation test for independent samples: Experiment 1: $n = 10$; Experiment 2: $n = 7$; $P = 0.04$).

480 During probe trials (reversal of visual cues), the mean number of correct choices (choice of the previously rewarded goal arm) made by cuttlefish ($34 \pm 12\%$) was significantly lower than at the end of acquisition ($100 \pm 0\%$; exact permutation test for paired samples: $n = 7$, $P = 0.016$; see Fig. 3a). Analyses of individual performances showed that five of the seven cuttlefish tested used a place strategy, choosing the previously unrewarded goal arm when the right/left positions of visual cues were reversed. The remaining two cuttlefish used a response strategy, choosing the previously rewarded goal arm despite the reversal of the visual cues. The number of sessions to acquisition was not different between place-learners (four sessions i.e. 20 trials) and response-learners (6 ± 1 sessions, i.e. 25–35 trials).

495 Although most cuttlefish appeared to rely on place learning to orient in the T-maze (five out of seven), the numbers of cuttlefish using the two strategies were not significantly different from chance. However, cuttlefish were significantly more likely to use the place learning strategy in Experiment 2 than they were in Experiment 1 (Fisher exact test: Experiment 1: $n = 10$; Experiment 2: $n = 7$; $P = 0.0175$).

Fig. 3 Experiment 2: **a** Mean percentages of correct choices per session (\pm SEM) during the first and last sessions of training and during the visual cues inversion probe trials. **b** Number of cuttlefish using the place or response arm during the visual cues inversion probe trials. The asterisk (*) indicates significant difference (exact permutation tests for paired samples: $P < 0.05$)



504 Experiment 2 showed that some cuttlefish oriented using place learning (five out of seven) while others oriented using response learning (two out of seven). In Experiment 2, two proximal visual cues were provided within an homogeneous environment (curtained area) whereas in Experiment 1, only distal visual cues were available. In each experiment, both place and response learning were observed, but which strategy was preferred appeared to depend on the salience of the visual stimuli available. Learning acquisition seemed more effective when visual cues were provided just above the apparatus.

515 General discussion

516 This study demonstrates that cuttlefish (1) exhibit a strong side-turning preference in a T-maze that can be reliably detected in a single probe trial (preliminary test), (2) can use response or place strategies when trained with a simple orientation task in a T-maze (Experiments 1 and 2), (3) rely preferentially on a response strategy rather than a place strategy when distal cues are provided around the maze (Experiment 1) and (4) are more likely to rely on place learning when visual cues (two geometric cues) are provided above water (Experiment 2).

526 The preliminary test showed that 11 out of the 15 cuttlefish displayed a pervasive side-turning preference. Significant side bias has been previously documented (Karson 2003; Karson et al. 2003); however, this current study is the first to document that such side bias can be reliably detected in a single probe trial. Such laterality could be the result of an eye use preference in cuttlefish, as has been suggested for octopus (Byrne et al. 2002). Such asymmetry could prevent a conflict in responding to the visual inputs of the two laterally placed eyes (Vallortigara et al. 1999). A population can be considered lateralised when more than 50% of the individuals are lateralised in the same direction (Lehman, 1981). Although 8 of 12 cuttlefish in this current

539 study had a preference for the right side, previous stud- 598
540 ies found a more even distribution of preferences (Karson 599
541 2003; Karson et al. 2003); thus, current evidence does not 600
542 support a population level bias. 601

543 Experiments 1 and 2 demonstrated that in a simple orienta- 602
544 tion task, cuttlefish are likely to use either a response 603
545 strategy or a place strategy (visual cues) to orient. How can 604
546 we explain their reliance on one strategy rather than the 605
547 other? 606

548 The most effective of these two strategies likely de- 607
549 pends, in part, on task demands (O'Keefe and Nadel, 608
550 1978). Cephalopods are known for their learning abili- 609
551 ties (Sanders 1975; Mather 1995; Hanlon and Messenger 610
552 1996); this simple two-choice discrimination problem was 611
553 well within their capabilities. In our experiments, clearly 612
554 both strategies were effective for solving the problem. Each 613
555 type of association (response and reward, or place and 614
556 reward) was learned by at least some of the cuttlefish. 615
557 Cuttlefish demonstrated their ability to use each strategy 616
558 efficiently. 617

559 The most effective strategy also likely depends on the 618
560 detectability and salience of available cues (Restle 1957; 619
561 Carman and Mactutus 2001). Cephalopods have well- 620
562 developed eyes (Budelmann 1994; Messenger 1968). The 621
563 optical problems of the refraction and image-distortion that 622
564 arise from surface ripples do not prevent the successful 623
565 capture of crabs that are presented above the surface of 624
566 the water (Boletzky 1972). Cuttlefish appear able to see 625
567 and discriminate objects presented above the surface of the 626
568 water. Even though visual cues were provided in both Ex- 627
569 periments 1 and 2, more cuttlefish used the place strategy 628
570 in Experiment 2. In Experiment 1, distal visual cues must 629
571 have appeared less salient for the cuttlefish, creating a ho- 630
572 mogeneous visual surrounding that greatly predisposed the 631
573 cuttlefish to learn response instead of place. Place learn- 632
574 ing was more common when the visual stimuli above the 633
575 maze were proximal and very different (striped rectangle 634
576 and spotted square), making them both highly detectable 635
577 and salient. 636

578 In Experiment 2, we did not find any difference in the 637
579 number of sessions to criterion between cuttlefish using 638
580 response and place strategies. Some studies of vertebrates 639
581 have shown that subjects using a place strategy reach a 640
582 learning criterion faster than those using a response strat- 641
583 egy (rats, O'Keefe and Nadel 1978); other studies did not 642
584 report any difference in the number of trials to acquisi- 643
585 tion (rats, Colombo et al. 2003; three-spined sticklebacks, 644
586 Odling-Smee and Braithwaite 2003; goldfish, López et al. 645
587 1999). Previous studies in vertebrates made clear that the 646
588 place and response learning occur simultaneously and in 647
589 parallel (Gibson and Shettleworth 2005), and suggested that 648
590 the relative dominance of place depends on the amount of 649
591 differential visual stimulation (Restle 1957). The dictates 650
592 of place and response learning conflicted in the probe trials 651
593 used in our experiments. A consistent arm choice based on 652
594 one strategy (response or place learning) did not exclude 653
595 the possibility that the other type of learning had occurred 654
596 at the same time. In this case, cuttlefish could have acquired 655
656 two redundant kinds of information. In their natural envi-

ronment, such parallel learning could allow cuttlefish to 598
599 shift quickly from one strategy to another if environmen- 600
601 tal conditions change. In further studies, we are exploring 602
603 this possibility, and are tracing the course of acquisition 604
605 to explore possible changes in the strengths of the two 606
607 strategies. 608

609 One of the purposes of our study was to develop a proto- 610
611 col to test spatial learning strategies in cuttlefish. Although 612
613 little is known about the behaviour of cuttlefish in their nat- 614
615 ural habitat, their survival may depend on adaptive skills 616
617 such as the ability to find and return to specific sites, such 618
619 as patches of food or places of safety. Protocols involving 620
621 substantial handling or food deprivation do not result in 622
623 reliable food-searching behaviour. Consequently, we chose 624
625 to develop a task requiring subjects to learn to find a place 626
627 of safety to escape an open and strongly lit area (as in Boal 628
629 et al. 2000; Karson 2003; Karson et al. 2003). Clearly, our 630
631 ability to understand the real implications of spatial learn- 632
633 ing abilities in cuttlefish would be considerably enhanced 634
635 if field data were available. 636

637 Our study demonstrates for the first time the presence of 638
639 multiple spatial learning strategies in cuttlefish that appear 640
641 to closely parallel those described in vertebrates. Consider- 642
643 ing differences in brain and sensory-motor structures of the 644
645 cuttlefish and arthropods or vertebrates, understanding the 646
647 mechanisms of spatial orientation in cuttlefish will provide 648
649 important tools for understanding the evolution of spatial 649
650 memory processes. 651

Acknowledgements We thank Dr. J. Lejeune for statistical advice 626
627 and the staff of the C.R.E.C. for their technical assistance. This 628
629 research was supported by a grant from the Ministère de la Recherche 630
631 et de la Technologie to C.A. The experiments complied with the 632
633 French animal testing laws. 634
635

631 References

- 632 Andrade C, Alwarshetty M, Sudha S, Suresh Chandra J (2001) Ef- 633
634 fect of innate direction bias on T-maze learning in rats: im- 635
636 plications for research. *J Neurosci Methods* 110:31–35 DOI 637
638 10.1016/S0165-0270(01)00415-0 639
640 Benhamou S, Poucet B (1996) A comparative analysis of spa- 641
642 tial memory processes. *Behav Processes* 35:113–126 DOI 643
644 10.1016/0376-6357(95)00060-7 645
646 Boal JG (1996) A review of simultaneous visual discrimination as a 647
648 method of training octopuses. *Biol Rev* 71:157–190 649
650 Boal JG, Dunham AW, Williams KT, Hanlon RT (2000) Experimen- 651
652 tal evidence for spatial learning in octopuses (*Octopus bimacu-* 653
654 *loides*). *J Comp Psychol* 114(3):246–252 655
656 Boletzky SV (1972) A note on aerial prey-capture by *Sepia officinalis* 657
658 (Mollusca, Cephalopoda). *Vie Milieu* 23(1A):133–140 659
660 Budelmann BU (1994) Cephalopod sense organs, nerves and the 661
662 brain: adaptations for high performance and life style. *Mar Fresh* 663
664 *Behav Physiol* 25:13–33 665
666 Byrne RA, Kuba M, Griebel U (2002) Lateral asymmetry of 667
668 eye use in *Octopus vulgaris*. *Anim Behav* 64:461–468 DOI 669
670 10.1006/anbe.2002.3089 671
672 Byrne RA, Kuba MJ, Meisel DV (2004) Lateralized eye use in *Oc-* 673
674 *topus vulgaris* shows antisymmetrical distribution. *Anim Behav* 675
676 68(5):1107–1114 DOI 10.1016/j.anbehav.2003.11.027 677
678 Carman HM, Mactutus CF (2001) Proximal versus distal cue utiliza- 679
680 tion in spatial navigation: the role of visual acuity?. *Neurobiol* 681
682 *Learn Mem* 78:332–346 DOI 10.1006/nlme.2002.4062 683

- 658 Colombo PJ, Brightwell JJ, Countryman RA (2003) Cognitive
659 strategy-specific increases in phosphorylated cAMP Response
660 Element-Binding protein and c-Fos in the hippocampus and dor-
661 sal striatum. *J Neurosci* 23(8):3547–3554
- 662 Dudchenko PA (2001) How do animals actually solve the T maze?.
663 *Behav Neurosci* 115(4):850-860
- 664 Franz MO, Mallot HA (2000) Biomimetic robot navigation. *Robot*
665 *Auton Syst* 30:133–153 DOI 10.1016/S0921-8890(99)00069-X
- 666 Gibson BM, Shettleworth SJ (2005) Place versus response learning
667 revisited: tests of blocking on the radial maze. *Behav Neurosci*
668 119(2):567–586 DOI 10.1037/0735-7044.119.2.567
- 669 Giurfa M, Capaldi EA (1999) Vectors, routes and maps: New discov-
670 eries about navigation in insects. *Trends Neurosci* 22:237–242
671 DOI 10.1016/S0166-2236(99)01406-X
- 672 Golledge RG (1999) Wayfinding behavior. Johns Hopkins University
673 Press, Baltimore
- 674 Hanlon RT, Messenger JB (1996) *Cephalopod Behaviour*. Cambridge
675 University Press, Cambridge, UK
- 676 Healy S (1998) *Spatial representation in animals*. Oxford University
677 Press, Oxford
- 678 Jacobs LF (2003) The evolution of the cognitive map. *Brain Behav*
679 *Evol* 62:128–139
- 680 Karson MA (2003) Simultaneous discrimination learning and its neu-
681 ral correlates in the cuttlefish *Sepia officinalis* (Cephalopoda:
682 Mollusca). Doctoral dissertation. Michigan State University,
683 East Lansing, MI
- 684 Karson MA, Boal JG, Hanlon RT (2003) Experimental evidence for
685 spatial learning in cuttlefish (*Sepia officinalis*). *J Comp Psychol*
686 117(2):149–155
- 687 Lehman RAW (1981) Lateralized asymmetry of behavior in animals
688 at the population and individual level. *Behav Brain Sci* 4:28
- 689 López JC, Broglio C, Rodríguez F, Thinus-Blanc C, Salas C (1999)
690 Multiple spatial learning strategies in goldfish (*Carassius auro-*
691 *tus*) *Anim Cogn* 2:109–120 DOI 10.1007/s100710050031
- Mather JA (1991) Navigation by spatial memory and use of visual
landmarks in octopuses. *J Comp Physiol A* 168:491–497
- Mather JA (1995) Cognition in cephalopods. In: Slater PJB, Rosen-
blatt JS, Snowdon CT (eds) *Advances in the study of behavior*
vol 24. Academic Press, San Diego, CA, US, pp 317–353
- Messenger JB (1968) The visual attack of the cuttlefish, *Sepia offic-*
inalis. *Anim Behav* 16:342–357
- Odling-Smee L, Braithwaite VA (2003) The influence of habi-
tat stability on landmark use during spatial learning in
the three-spined stickleback. *Anim Behav* 65:701–707 DOI
10.1006/anbe.2003.2082
- O’Keefe J, Nadel L (1978) *The hippocampus as a cognitive map*.
Oxford University Press, Oxford
- Packard A (1972) Cephalopods and fish: the limits of convergence.
Biol Rev 47:241–307
- Packard MG, McGaugh JL (1996) Inactivation of hippocampus or
caudate nucleus with lidocaine differentially affects expression
of place and response learning. *Neurobiol Learn Mem* 65(1):65–
72 DOI 10.1006/nlme.1996.0007
- Restle F (1957) Discrimination of cues in mazes: A resolution of the
“place-vs-response” question. *Psychol Rev* 64:217–228
- Sanders GD (1975) *The Cephalopods*. In: Corning WC, Dyal JA,
Willows AOD (eds) *Invertebrate learning* vol 3. Plenum Press,
New York, pp 1–101
- Siegel S, Castellan NJ (1988) *Nonparametric statistics for the behav-*
ioral sciences, 2nd edn. McGraw-Hill, New York
- Vallortigara G, Rogers LJ, Bisazza A (1999) Possible evolution-
ary origins of cognitive brain lateralization. *Brain Res Rev*
30(2):164–175 DOI 10.1016/S0165-0173(99)00012-0
- Walker JJ, Longo N, Bitterman ME (1970) The octopus in the lab-
oratory. Handling, maintenance, training. *Behav Res Methods*
Instrum 2(1):15–18
- Wehner R, Lehrer M, Harvey WR (1996) *Navigation: migration and*
homing, vol 199. Company of Biologists Limited, Cambridge
- Wells MJ (1964) Detour experiments with octopuses. *J Exp Biol*
41:621–642