Telencephalon ablation impairs goldfish allocentric spatial learning in a "hole-board" task

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The present work analyzes the involvement of telencephalon of goldfish in spatial strategies, using a procedure analogue to the hole-board task. With this aim, goldfish with sham operation or telencephalon ablation were trained to find a baited feeder within a twenty-five feeder matrix, which maintained stable spatial relationships relative to five peripheral landmarks. After training, different types of probe tests were conducted. Although both groups learned the task, probe trials showed that whereas the sham animals used the whole configuration of cues to implement map-like strategies the telencephalic animals used a guidance strategy based on cues located in the vicinity of the baited feeder. These results confirm the role of teleost fish telencephalon in the use of allocentric strategies obtained with other spatial procedures, and indicate that the hole-board task described here for goldfish is a useful tool to assess the neural bases of spatial cognition in teleost fish.

Key words: hole-board task, cue navigation, place navigation, spatial learning, telencephalon, teleost fish

Comparative studies have reported that the spatial learning and memory capabilities of teleost fish are remarkably similar to those observed in land vertebrates. Fish can use a variety of spatial navigation strategies subserved by different cognitive systems that are supported by well-differentiated neural substrates. For example, besides cue learning and other body-centered egocentric mechanisms, fish can use map-like, relational representations of the environment, that seem to be based on the simultaneous processing and encoding of the relationships among multiple spatial landmarks, and their metrical and geometrical properties (Rodríguez et al. 1994, 2002, Broglio et al. 2003, Vargas et al. 2004, Salas et al. 2006, Sovrano et al. 2007).

In amniotes, spatial strategies based on map-like representations depend on the hippocampal formation and related structures (O'Keefe and Nadel 1978, Burgess et al. 1999). Specifically, lesions to the hippocampus of mammals, birds and reptiles, produce selective deficits in spatial tasks that require the encoding of relationships among multiple environmental features, but not in tasks that require approaching a single cue (Morris et al. 1982, Jarrard et al. 1984, Schenk and Morris 1985, Eichenbaum et al. 1990, Day et al. 2001, López et al. 2003, Tommasi et al. 2003, Hampton et al. 2004).

In teleost fish, an anamniotic vertebrate group, the telencephalon seems to be essential for allocentric spatial memory. Previous studies have shown that telencephalic ablation in fish impairs the use of spatial strategies based on map-like, relational memory, but it does not impair, or even facilitates, the use of egocentric strategies (Salas et al. 1996a,b, López et al. 2000, Rodríguez et al. 2002). For example, López and coauthors (2000) reported that both, intact and telencephalon-ablated goldfish trained in a mixed place-cue procedure learned the task with accuracy. However, the probe tests in which choices based on a place strategy were dissociated from those based on a guidance strategy revealed that whereas the intact goldfish could use both types of strategies, the telencephalon-ablated

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goldfish used exclusively a guidance strategy, based on approaching a single cue. These spatial deficits in goldfish resemble those seen after hippocampal damage in amniotes.

The aim of the present experiment was to further investigate the range of spatial strategies that can be used by goldfish to find a goal in the space, using a new procedure analogue of the hole-board task. With this purpose, telencephalon ablated and sham goldfish were trained to locate a baited feeder within a twenty five-feeder matrix, surrounded by five visual cues. Subsequent probe trials were performed to analyse the strategies used by the animals to reach the goal.

In this experiment 16 goldfish (*Carassius auratus*), 12–14 cm in length, were used. They were maintained for two months prior to the experiment in 200 l glass aquaria with aerated and filtered water at 20 ± 2°C on a 14/10 h light/dark cycle, and were fed once a day (Tetra pond, Ulrich Baemsch). Throughout the experiment the fishes only consumed the food-sticks they obtained in the daily session. Before the experiment the animals were randomly assigned to two different lesion groups: sham operation (Sh; *n*=8) and telencephalon ablation (Tel; *n*=8). The telencephalic ablation was performed by aspiration according to the methods described previously (Salas et al. 1996a,b). Briefly, the animals were anaesthetized by immersion in a tricaine methanesulfonate solution (0.02%) and subsequently were immobilized in a surgical chamber. An adjustable tube was introduced into the mouth of the animal to ensure a constant flow of aerated water through the gills. The concentration of anaesthetic in the water was kept at 0.02% during the surgical intervention. The cephalic skin and a section of the dorsal skull were removed carefully under visual control by means of a binocular microscope, and the fatty tissue inside was aspirated to expose the brain. Both telencephalic lobes were aspirated carefully with a glass micropipette connected to a manual vacuum system. After ablation, the piece of skull was replaced in its original position, fixed with cyanocrilate glue and covered with dental cement. Surgery for animals in the sham group was performed in the same way, except that the nervous tissue was not injured. After surgery the animals were returned to their home aquarium and allowed a recovery period of four days. Animal care protocols were approved by the European Directive (86/609/EEC).

The experimental apparatus was a circular tank (140 cm in diameter, 50 cm high) made out of white
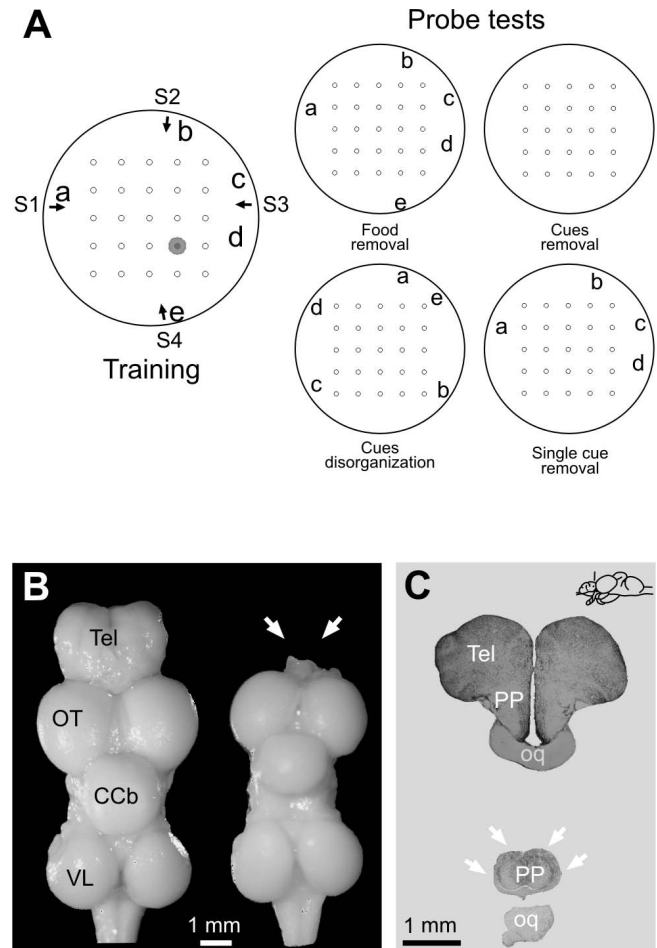

Fig. 1. Procedures. (A) Schematic representation of the apparatus and procedure used during training, and the four probe tests conducted in this experiment. The diagrams show a view of the experimental apparatus with the feeder matrix and the arrangement of the landmarks. The grey filled circle indicates the goal. Empty circles signal the location of the non baited feeders. a, b, c, d and e indicate the position of the five experimental cues. S1, S2, S3 and S4 indicate the four different starting points used during training. Only the single cue removal test for the landmark e is shown (the landmarks a, b, c, d were also removed in additional single cue removal test trials). (B and C) Telencephalic ablation extent. (B) Dorsal view of the brain of a sham animal (left) and of a telencephalon ablated one (right). (C). Transverse Nissl stained post-commissural section of the brain of a sham animal (up) and of a telencepahlon ablated one (down). The arrows indicate the boundary of the telencephalic tissue removal. (CCb) corpus cerebelli; (oc) optic chiasm; (OT) optic tectum; (PP) periventricular preoptic nucleus; (Tel) telencephalon; (VL) vagal lobe.

flexible vinyl filled with aerated water to a depth of 18 cm. Twenty-five feeders, equidistantly distributed to form a square matrix (80 cm in length) were positioned on the floor of the tank such that the central feeder in the matrix coincided with the topological centre of the maze (Fig. 1A). Each feeder consisted of an opaque glass cup (4.5 cm in diameter, 4.5 cm high), enclosing a latex tube (1.5 cm long) that enabled the experimenter to hide a food stick or a fake one. The fake sticks were made out of light brown latex and their appearance was very similar to that of the real food. The animals could remove the food by applying suction to the latex tube, but could not remove the false sticks since they were glued to this tube. Five different objects, placed around the feeder matrix and maintaining stable spatial relationships relative to the goal, served as landmarks (Fig. 1A). The objects were as follows; cue a, a plastic grey disc (25 cm in diameter); cue b, a blue plastic litter bin (20 cm in diameter × 30 cm in height); cue c, a plastic red funnel (20 cm in diameter on the base); cue d, a grey plastic vessel (20 cm in diameter × 35 cm in height); cue e, a yellow plastic box (20 × 20 × 10 cm). To exclude the possible use of extramaze cues to solve the task, grey curtains hanging from the ceiling to the floor surrounded the maze. A square array of four 60W fluorescent lights placed on the ceiling provided diffuse illumination to the maze. A video camera was used to monitor the behavior of the animals.

Prior to training, the fish were habituated to eat from the experimental feeders in a small aquarium. Then, they were individually habituated to retrieve food in the experimental maze. In these sessions the visual cues were not present, and only 6 of the 25 feeders, chosen at random, were baited. After this habituation phase the animals were deprived of food for two days before the experiment proper.

During the training only one of the feeders was baited (goal). The task consisted of learning the goal location, which maintained stable spatial relationships relative to the five experimental visual cues (Fig. 1A). The fishes were individually trained in daily 6-trial sessions. To begin each trial, the goldfish was released facing the inner wall of the tank. An error was recorded each time the animal sucked one of the twenty four unbaited feeders and a correct choice when the animal got the food stick. The time to obtain the reward was also recorded. The trial ended when the fish obtained the food or 2 min had

elapsed. Then, the animal was returned to the home aquarium for a 20 min intertrial interval. To eliminate the possible use of uncontrolled cues, the whole configuration formed by the feeder matrix and the five experimental cues were rotated twice on each training session (six rotations were used 0°, 60°, 120°, 180°, 240° and 300°). Four different start positions were used in a pseudorandom order (Fig. 1A).

Four different types of probe tests were conduced to analyze the strategies used by the fish to locate the goal (Fig. 1A). The apparatus and the general procedures remained unchanged during probe trials, except that all the feeders had a fake food stick. A food removal test was performed to elucidate whether the animals could identify the correct feeder by olfactory or visual cues. A cues removal test consisted of the removal of all the visual cues simultaneously to test whether the animals used them to locate the goal. In the cues disorganization test the habitual cues configuration was disarranged; thus, although all the experimental cues were present, the spatial relationships among themselves and relative to the goal were altered. This probe test evaluated the role of the global configuration of the cues for locating the correct feeder. In the single cue removal test one individual cue was removed at a time. This test evaluated whether the animals used any particular cue as a landmark to locate the goal. Two trials for each probe test were conduced for each animal, except for the single cue removal test; in this case, five different trials were conduced, one for each cue. The probe trials were interspersed in a pseudorandom order once the learning criterion was reached. No more than two probe trials were included per session and at least two training trials were conduced between any two probe trials. The probe trial ended when 1 min had elapsed. All the choices made during this period were recorded.

After completion of the experiment, the histological analysis showed that the ablated animals exhibited a complete removal of the telencephalic tissue, whereas the preoptic areas and optic tracts were not injured (Fig. 1B and 1C).

To evaluate the performance of goldfish, the number of errors and the time to find the goal were analysed. In addition, the efficiency of the goldfish search behaviour was evaluated by means of the spatial accuracy index (SAI). The SAI was calculated on the basis of the distance of the responses to the goal assigning a value, ranging from 0 to 1, to each feeder, inversely

proportional to the distance to the goal (1 for the correct feeder and 0 for the most distant ones; see insert in Fig. 2A). In each trial, the SAI represents the mean
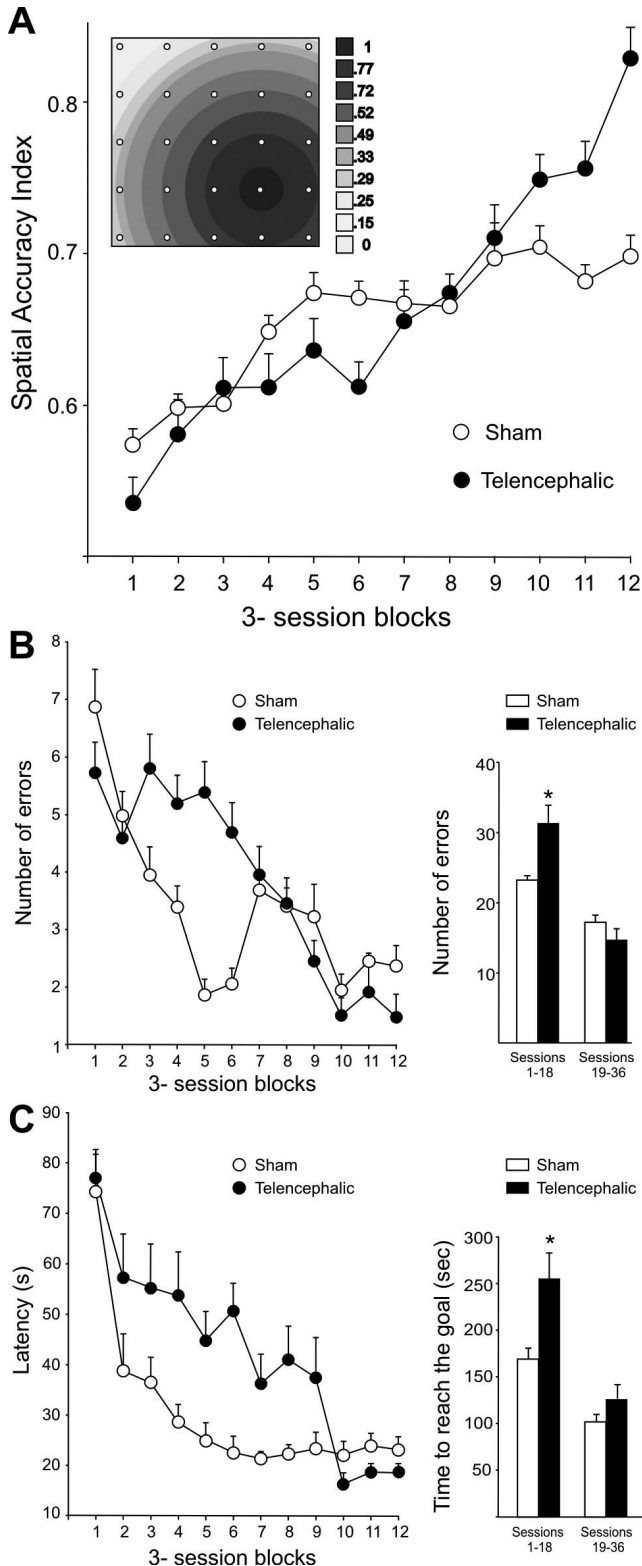
of the spatial accuracy values of the feeders chosen by the animal. The learning criterion consisted of 2 out of 3 consecutive sessions with SAI scores higher than 0.7. The data obtained during training were grouped in blocks of three sessions (18 trials).

The performance of the animals during training is shown in Figure 2. At the beginning of training, session block 1, both groups exhibited a low accuracy to locate the goal, as indicated by their SAI values (Sh: $\bar{x}=0.57$, $\sigma=0.01$; Tel: $\bar{x}=0.54$, $\sigma=0.01$), the number of errors (Sh: $\bar{x}=6.87$, $\sigma=0.65$; Tel: $\bar{x}=5.72$, $\sigma=0.53$), and the latency to reach the goal (Sh: $\bar{x}=74.3$, $\sigma=7.39$; Tel: $\bar{x}=77.09$, $\sigma=5.65$, see Fig. 2), showing no statistically significant between-group differences in any of these measures. Along training, session blocks 1–12, the animals in both groups progressively improved their accuracy to solve the task, as indicated by the statistically significant increase observed in the SAI values (Friedman, $\chi^2_{(11)}=52.5$ and $\chi^2_{(11)}=71.02$, both $Ps=0$, for Sh and Tel groups, respectively), and the significant decrease in the number of errors (Friedman, $\chi^2_{(11)}=53.06$ and $\chi^2_{(11)}=55.89$, both $Ps=0$, for the Sh and Tel groups, respectively), and in the time to reach the goal (Friedman, $\chi^2_{(11)}=37.96$ and $\chi^2_{(11)}=58.92$, both $Ps=0$, for Sh and Tel groups, respectively). In addition, no differences were found in the acquisition of the learning criterion, which was reached in session 9 by the animals in both groups. The analysis of the accumulated values of the number of errors and the time to reach the goal, revealed that in the first half of training the ablated group accumulated more errors and invested more time in localizing the reinforced feeder than the sham group (Mann-Whitney, $U=11$ and $U=7$, both $Ps<0.028$, for number of errors and latency, respectively; see Fig. 2B and 2C). In contrast, at the end of

Fig. 2. Performance of the sham and telencephalon ablated groups during training. (A). Mean spatial accuracy index (SAI) values for each group during training sessions. Error bars denote SEM. In the insert (top, left), the rings in different shades of grey represent distance to the goal. Note that the feeders included in each ring present the same spatial value. The values range from 1 for the goal (feeder in the black circle) to 0 for the most distant feeder (feeder in the pale grey ring). (B) Mean number of errors and (C) time to reach the goal during training for both groups. The histograms represent the accumulated number of errors and the accumulated time to reach the goal, during sessions 1–18, and sessions 19–36 of training. Error bars denote SEM. Asterisks (*) denote significant between-group differences ($P<0.05$).
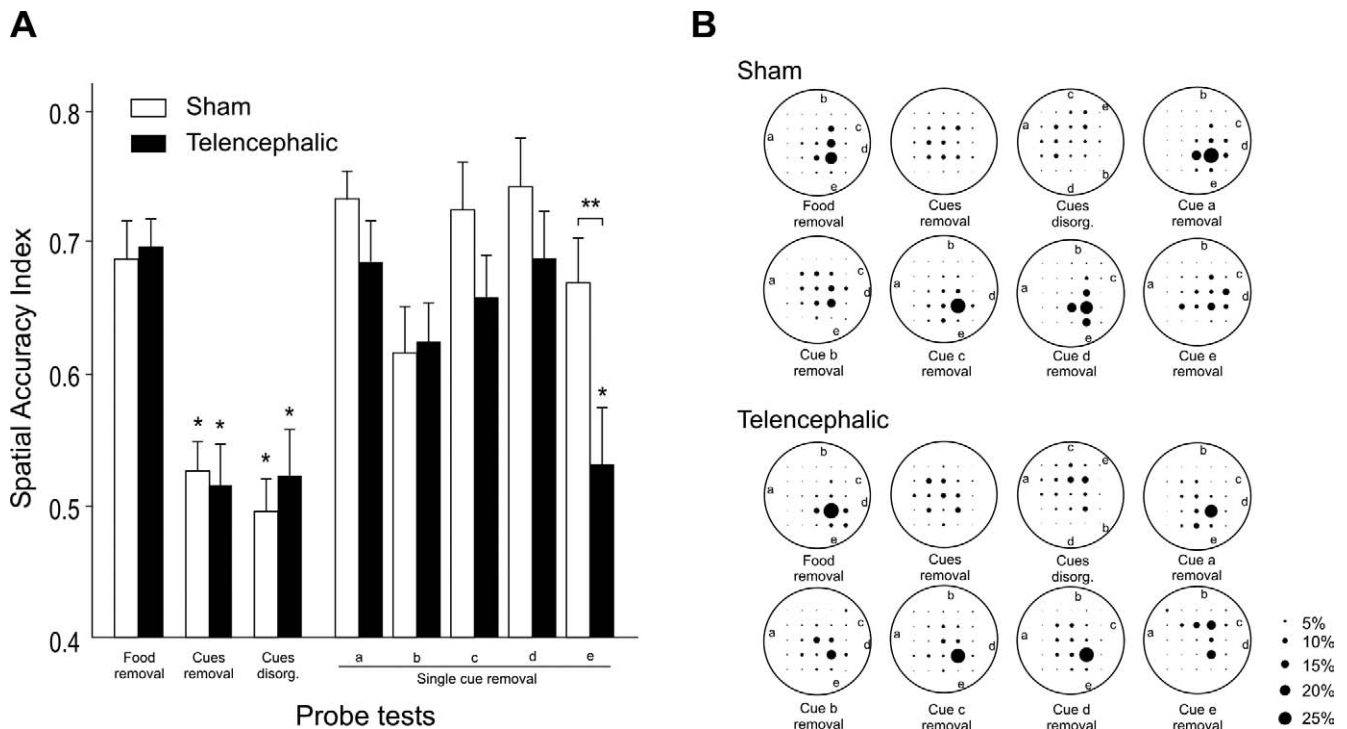
A

B

Fig. 3. Performance of the sham and telencephalon ablated groups in the different probe tests. (A) Mean spatial accuracy index (SAI) values. Error bars denote SEM. Asterisks (*) denote significant intra-group differences ($P<0.05$). Double asterisk (**) denotes significant between-group differences ($P<0.05$). (B) Patterns of choice distribution. The diameter of each point denotes the percentage of choice.

the training, the level of performance of the ablated animals surpassed that exhibited by the sham group. In fact, the ablated animals obtained higher SAI values than the sham animals in the session blocks 8 to 12, reaching statistically significant differences on the two last session blocks (Bonferroni Mann-Whitney, $U=8$ and $U=0$, both $Ps \leq 0.01$, for session blocks 11 and 12, respectively, Fig. 2A).

Figure 3 summarizes the data obtained in the probe tests. In the food removal test, the level of accuracy shown by the animals in both groups was similar to that achieved on the last three training sessions previous to the presentation of the probe trials, so these SAI values were used as baseline to analyse the results of the remaining probe tests. In addition, no statistically significant between-group differences were found in the SAI values for the food removal test, indicating that the ability of the animals to locate the goal was not a consequence of the odour or direct visualization of the reward. Cues manipulation in either, the cues removal or the cues disorganization tests had similar effects for both groups. Therefore, a significant decrease in the SAI values relative to their respective

baselines was observed in both groups in the cues removal test (Wilcoxon, $Z=-2.52$ and $Z=-2.83$, both $Ps \leq 0.017$, for Sh and Tel groups, respectively), and in the cues disorganization test (Wilcoxon, $Z=-2.52$ and $Z=-2.24$, both $Ps \leq 0.025$ for the Sh and the Tel groups, respectively). In addition, no statistically significant between-group differences were observed in these tests (Fig. 3). However, important differences were found between the sham and the telencephalon ablated groups in the single cue removal test. Whereas the SAI values in the sham animals did not differ from that of the baseline in any of these probe trials, the removal of the cue e caused a significant decrease in the SAI values of the ablated animals, relative to their baseline (Wilcoxon, $Z=-2.1$, $P=0.036$, Fig. 3), and to the sham group (Mann-Whitney, $U=12$, $P=0.038$). Interestingly, statistically significant differences were not observed in the remaining single cue removal probe trials for the ablated animals, neither relative to their baseline, nor relative to the sham animals (Fig. 3).

The most important result of the present experiment is that the ablation of the telencephalon selectively impairs goldfish to implement allocentric spatial strat-

egies. In fact, goldfish with telencephalon ablation, in contrast to the sham animals, were unable to navigate accurately to the goal using the arrangement of the experimental landmarks in a map-like representation. Although both sham and telencephalon ablated goldfish, learned to identify the location of a baited feeder within a twenty five-feeder matrix on basis of the information provided by the visual cues, their performance in some critical probe trials indicates that the nature of the navigational strategies used by the two groups was different. In this regard, the single cue removal test revealed that the accuracy of the sham animals to locate the goal after the elimination of any one of the single cue, was based on the general spatial arrangement maintained by the remaining cues. These data indicate that the sham fish used place strategies based on a map-like representation of the environment. In addition, the results in the cue disorganization test provide additional evidence concerning the use of allocentric representations by the sham fish. Thus, the deficit of these animals to reach the goal when the topographical relationships among the different cues were modified indicates that the relevance of the spatial information was dependent on the stability of the spatial distribution of the landmarks. In fact, the deficit observed for the sham fish in this test was as severe as that observed for the same animals in the cues removal test, when all the cues were eliminated. Landmark stability and resistance to a partial loss of relevant spatial information of the environment are distinctive features of map-like or relational representations (O'Keefe and Nadel 1978, Mazmanian and Roberts 1983, Thinus-Blanc 1996, Jacobs et al. 1998). In contrast, the impairment observed in the telencephalon ablated goldfish when one of the cues placed close to the goal (cue e) was removed, indicates that these animals located the goal by approaching this particular landmark. This result suggests that the ablated fish used a cue navigation strategy to find the goal. Thus, the fact that the ablated, but not the sham animals, used guidance strategies strongly suggests that the telencephalon ablation disrupts the ability of the teleost fish to encode the reciprocal spatial relationships between the experimental landmarks and the goal, impairing the implementation of allocentric frames of spatial representation.

The findings of the present work are consistent with those obtained in previous experiments in which telencephalon ablated goldfish trained in other spatial procedures showed a dramatic spatial cognition deficit (Salas et al. 1996a,b, 2006, López et al. 2000, Rodríguez et al. 2002). The present results, obtained in a notably different spatial task, are particularly relevant, as they provide convergent evidence on the presence of multiple spatial memory systems in teleost fish, which depend on separated neural circuits (Rodríguez et al. 1994, 2002, Salas et al. 1996a,b, 2003, 2006). The spatial deficits observed after telencephalon ablation in this experiment are also consistent with those reported in hippocampal damaged mammals, birds and reptiles trained in similar place tasks (Gaffan and Harrison 1989, Morris et al. 1982, Rodríguez et al. 2002, López et al. 2003, Hampton et al. 2004, Vargas et al. 2004, Cheng et al. 2006).

These results, obtained with a new procedure, an analogue of the hole-board task, increase the validity and the comparative value of the data concerning spatial cognition and its neural basis in fishes. In addition, the similarity of these data with previous experiments in which telencephalon ablated goldfish were trained in other spatial procedures indicates that the hole-board analogue task described here may become a useful experimental instrument to analyse the neural substrate of spatial learning and memory in fishes.

Broglio C, Rodríguez F, Salas C (2003) Spatial cognition and its neural basis in teleost fish. Fish Fisher 4: 247–255.

Burgess N, Jeffery KJ, O'Keefe J (1999) The Hippocampal and Parietal Foundations of Spatial Cognition. Oxford University Press, London.

Cheng K, Spetch ML, Kelly DM, Bingman VP (2006) Small-scale spatial cognition in pigeons. Behav Processes 72: 115–127.

Day LB, Crews D, Wilczynski W (2001) Effects of medial and dorsal cortex lesions on spatial memory in lizards. Behav Brain Res 118: 27–42.

Eichenbaum H, Stewart C, Morris RG (1990) Hippocampal representation in place learning. J Neurosci 10: 3531–3542.

Gaffan D, Harrison S (1989) Place memory and scene memory: Effects of fornix transection in the monkey. Exp Brain Res 74: 202–212.

Hampton RR, Hampstead BM, Murray EA (2004) Selective hippocampal damage in rhesus monkeys impairs spatial memory in an open-field test. Hippocampus 14: 808–818.

Jacobs WH, Thomas KGF, Laurence HE, Nadel L (1998) Place learning in virtual space. II. Topographical relations as one dimension of stimulus control. Learn Motiv 29: 288–308.

Jarrard LE, Okaichi H, Goldschmidt R, Stewart O (1984) On the role of the hippocampal connections in the performance of place and cue tasks: comparison with damage to the hippocampus. Behav Neurosci 98: 946–954.

López JC, Bingman VP, Rodríguez F, Gómez Y, Salas C (2000) Dissociation of place and cue learning by telencephalic ablation in goldfish. Behav Neurosci 114: 687–699.

López JC, Vargas JP, Gómez Y, Salas C (2003) Spatial and non-spatial learning in turtles: the role of medial cortex. Behav Brain Res 143: 109–120.

Mazmanian DS, Roberts WA (1983) Spatial memory in rats under restricted viewing conditions. Learn Motiv 14: 123–139.

Morris RG, Garrud P, Rawlins JNP, O´Keefe J (1982) Place navigation impaired in rats with hippocampal lesions. Nature 297: 681–683.

O'Keefe J, Nadel L (1978) The Hippocampus as a Cognitive Map. Oxford University Press, London.

Rodríguez F, Durán E, Vargas JP, Torres B, Salas C (1994) Performance of goldfish trained in allocentric and egocentric maze procedures suggests the presence of a cognitive mapping system in fishes. Anim Learn Behav 22: 409–420.

Rodríguez F, López JC, Vargas JP, Gómez Y, Broglio C, Salas C (2002) Conservation of spatial memory function in the pallial forebrain of amniotes and ray finned- fishes. J Neurosci 22: 2894–2903.

Salas C, Broglio C, Rodríguez F, López JC, Portavella M, Torres B (1996a) Telencephalic ablation in goldfish impairs performance in a spatial constancy problem but not in a cued one. Behav Brain Res 79: 193–200.

Salas C, Rodríguez F, Vargas JP, Durán E, Torres B (1996b) Spatial learning and memory deficits after telencephalic ablation in goldfish trained in place and turn maze procedures. Behav Neurosci 110: 1–16.

Salas C, Broglio C, Durán E, Gómez A, Ocaña FM, Jiménez-Moya F, Rodríguez F (2006) Neuropsychology of learning and memory in teleost fish. Zebrafish 3: 157–171.

Salas C, Broglio C, Rodríguez F (2003) Evolution of forebrain and spatial cognition in vertebrates: Conservation across diversity. Brain Behav Evol 62: 72–82.

Schenk F, Morris RG (1985) Dissociation between components of spatial memory in rats after recovery from the effects of retrohippocampal lesions. Exp Brain Res 58: 11–28.

Sovrano VA, Bisazza A, Vallortigara G (2007) How fish do geometry in large and in small spaces. Anim Cog 10: 47–54.

Thinus-Blanc C (1996) Animal Spatial Cognition. Behavioral and Neural Approaches. World Scientific, UK.

Tommasi L, Gagliardo A, Andrew RJ, Vallortigara G (2003) Separate processing mechanisms for encoding of geometric and landmark in formation in the avian hippocampus. Eur J Neurosci 17: 1695–1700.

Vargas JP, Lopez JC, Salas C, Thinus-Blanc C (2004) Encoding of geometric and featural spatial information by goldfish (*Carassius auratus*). J Comp Psychol 118: 206–216.