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Emotional and spatial learning in goldfish is dependent on different telencephalic pallial systems

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Abstract

In mammals, the amygdala and the hippocampus are involved in different aspects of learning. Whereas the amygdala complex is involved in emotional learning, the hippocampus plays a critical role in spatial and contextual learning. In fish, it has been suggested that the medial and lateral region of the telencephalic pallia might be the homologous neural structure to the mammalian amygdala and hippocampus, respectively. Although there is evidence of the implication of medial and lateral pallium in several learning processes, it remains unclear whether both pallial areas are involved distinctively in different learning processes. To address this issue, we examined the effect of selective ablation of the medial and lateral pallium on both two-way avoidance and reversal spatial learning in goldfish. The results showed that medial pallium lesions selectively impaired the two-way avoidance task. In contrast, lateral pallium ablations impaired the spatial task without affecting the avoidance performance. These results indicate that the medial and lateral pallia in fish are functionally different and necessary for emotional and spatial learning, respectively. Present data could support the hypothesis that a sketch of these regions of the limbic system, and their associated functions, were present in the common ancestor of fish and terrestrial vertebrates 400 million years ago.

Introduction

In mammals, the existence of different telencephalon-based learning systems, that comprise the hippocampus and the amygdala, has been proposed (Schacter & Tulving, 1994). It is commonly agreed that the hippocampus is involved in spatial, relational and/or contextual learning (O'Keefe & Nadel, 1978; Burgess *et al*., 1999; Eichenbaum *et al*., 1999; Jeffery *et al*., 2004), whereas the amygdala is required for emotional learning (Gallagher & Chiba, 1996; Davis, 1992; LeDoux, 1995). Notably, studies on birds and reptiles, also, suggest the presence of these systems (Kling & Brothers, 1992; Sherry & Duff, 1996; Bingman *et al*., 1998; López *et al*., 2001), based on in the assumption that both the amygdala and hippocampus arise from homologous structures in all terrestrial vertebrates (Northcutt, 1981; Ulinski, 1990; Nieuwenhuys *et al*., 1998). In fish, most of our knowledge of the involvement of the telencephalon in behavioural processes comes from studies in which the entire telencephalon was ablated (Hollis & Overmier, 1982; Overmier & Curnow, 1969). Importantly, this kind of lesion impairs the performance of aggressive and reproductive behaviours (Segaar & Nieuwenhuys, 1963; Overmier & Gross, 1974; Shapiro *et al*., 1974; de Bruin, 1980), as well as diverse types of learning, such as two-way active avoidance and spatial learning (López *et al*., 2000a,b; Overmier & Papini, 1985, 1986; Papini, 1985; Overmier & Hollis, 1990; Salas *et al*., 1996a,b; Portavella *et al*., 2003). However, these effects could be considered a sum of the impairments of several specialized telencephalon-based behavioural systems instead of unspecific effects of the lesion on an undifferentiated brain-system.

Because of the peculiar process of eversion that the telencephalic vesicle undergoes in actynopterigian fish (in contrast to the evagination that occurs in land vertebrates), it is difficult to establish homologies between the pallial areas of both fish and land vertebrates (Braford, 1995; Nieuwenhuys, 1963; Northcutt & Braford, 1980; Nieuwenhuys & Meek, 1990; Northcutt, 1995; Butler, 2000) (Fig. 1). Yet, past and recent neuroanatomical evidences suggest that the lateral and medial pallia of fish might be homologous to the hippocampus and pallial amygdala of land vertebrates, respectively (Braford, 1995; Northcutt & Braford, 1980; Nieuwenhuys & Meek, 1990; Northcutt, 1995; Butler, 2000). To test whether these anatomical hypotheses are supported by behavioural evidences, the following findings have been recently reported in goldfish: (i) ablation of the lateral telencephalic pallium impairs spatial learning in a T-maze procedure (Rodríguez *et al*., 2002); (ii), ablation of the medial pallium impairs learning in a two-way avoidance task (Portavella *et al*., 2004a,b); and (iii), ablation of the medial and lateral pallium generates differential effects on the memory, depending on the interstimuli gap of the avoidance tasks (Portavella *et al*., 2004a). These findings suggest the existence of different learning systems in fish, the neural substrates of which might be in different pallial telencephalic areas, consistent with the neuroanatomical proposal. However, these experiments did not evaluate if goldfish with medial and lateral lesions show selective deficits in emotional and spatial learning processes, respectively. The aims of this study were to test the specific effects of these lesions on different learning capacities and to explore the existence of differentiated learning and memory systems in fish.

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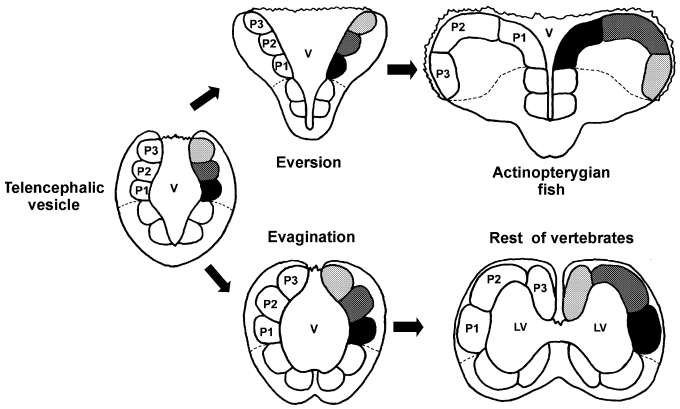

FIG. 1. Schematic representation of the two different processes of embryonic development of the telencephalic vesicle in vertebrates. In actinopterygian fish, an eversion phenomenon is produced, where the pallial region curves laterally, generating two telencephalic hemispheres with a single ventricular cavity dorsally disposed upon the hemispheres. In contrast, in the rest of vertebrates a process of evagination occurs. In this case, the pallial wall curves inside the middle line of the telencephalic vesicle and produces two hemispheres with an internal ventricular cavity in each one. P1, P2 and P3 are the three major divisions of the pallium. Based on Braford (1995); Butler (2000); Nieuwenhuys (1963); Nieuwenhuys & Meek (1990); Northcutt (1995); Northcutt & Braford (1980).

Materials and methods

Subjects and general procedure

Twenty-four experimentally naive goldfish, purchased from a local aquarium in Seville, served as subjects in this study. The fish were 10–12 cm in body length and lived in small groups in 200 L glass aquaria with aerated and filtered water kept at a constant temperature of 22 °C. The aquarium room was subject to a 14 : 10 h light : dark cycle (light from 07.00 h to 21.00 h). Pellets of dry food (Tetra-Pond; Ulrich Baemsch GmbH, Melle, Germany) were provided *ad libitum* daily.

The fish were divided into three experimental groups depending on the surgical procedure: LP (lateral pallium lesion); MP (medial pallium lesion); and control. Before training, animals from the MP group were submitted to ablation of the medial pallium ($n = 8$) and animals from the LP group to ablation of the lateral pallium ($n = 8$). The control group ($n = 8$) comprised four animals that did not receive any surgical intervention and four sham-operated animals that received the same surgery as the MP and LP groups but were not lesioned. Before training, in two procedures, every fish was anaesthetized by immersion in a solution of tricaine methansulphonate (MS222, Sigma, St. Louis, MO, USA: 1 : 20 000 p/v). The animal was then placed in the surgical chamber, where it remained fixed in place by lateral holders and partially bathed in water. A tube was inserted into the animal's mouth and connected to a pump that provided a constant flow of water with a constant concentration of anaesthetic through the gills during surgery. The dorsal skin and skull were carefully removed, and the underlying fatty tissue was removed by aspiration. The telencephalic tissue was aspirated carefully with a micropipette connected to a manual vacuum system. Surgery was performed under visual inspection by means of a binocular microscope. The sulcus limitans telencephali, sulcus lateralis, sulcus ypsiliformis, and anterior commissure were used as anatomical references to determine the location and extent of the neural tissue to be removed. Finally, the piece of skull was replaced in its original position and fixed with cyanocrylate glue. The fish was returned to its home tank for a recovery period of 5 days. Sham operations were performed exactly as described, except that the nervous tissue was not extracted. The control group did not receive any surgical intervention or anaesthetic. At the end of the experiment, the fish with telencephalic lesions and sham operations were deeply anaesthetized (1 : 5000 p/v) and perfused with 50 mL 0.9% saline solution, followed by 125 mL fixative solution (10% formalin in phosphate buffer, 0.1 M, pH 7.4). The brain was removed from the skull, inspected for a preliminary evaluation of the ablation, and cut in transversal sections (50-μm-thick) for histological analysis. Five days after surgery, all experimental fish were trained in both avoidance and spatial procedures. Every group was randomly subdivided in two subgroups. Each subgroup was first trained in a different procedure in a counterbalanced way.

The lesion's extent was valuated by means of reconstruction of microphotograph of the injured area on the atlas plates adapted from Peter & Gill (1975), and analysed by graphical software.

The experimental manipulations described in this manuscript were conducted in accordance with the Directive 86/609/CEE of the European Community Council and the Spanish Real Decreto 223/1988.

Two-way avoidance task

Apparatus

Four similar shuttle-boxes (see Portavella *et al*., 2004a) were used following an established design (Horner *et al*., 1961). Each shuttle box consisted of a water-filled glass tank (50 × 25 × 14 cm). Black PVC covered each long side, the floor was covered with white PVC, and the two box ends were clear and translucent to permit the green light presentation as the discriminative stimulus (10 W, 220 V AC, 50 Hz). On each long side, two stainless steel bars attached to metal plates were used as electrodes to deliver a uniform, mild electric shock as an aversive stimulus (0.39 V/cm, 50 Hz, pulsed 200 ms on and 800 ms off). A trapezoidal barrier (7.5 cm high, 10 cm wide at the top and 18 cm wide at the bottom) divided the shuttle box into two compartments. Over the barrier, two pairs of photoemitters (red lights; 24 V CC, 0.3 W) and photoreceptors (photoresistors; 3 V CC) detected the fish shuttle responses across it. The water level over the barrier was kept constant at 2 cm, giving a water level of 9.5 cm in each of the compartments. The water was aerated continuously. The shuttle boxes were controlled by a computerized system (Letica S.L) for rat conditioning, adapted and modified in our laboratory. The software driving the shuttle boxes (Skinner, Cibertec S.A) was also adapted to deliver the stimuli and to record the fish responses.

Avoidance task procedure

Before the learning sessions, fish were pre-exposed individually to the experimental apparatus during three consecutive sessions. On the first day, the animals were placed in the shuttle box with the water level set at 6 cm above the barrier, and were allowed to swim freely through the apparatus for a period of 30 min without any stimulus presentation. For the following two sessions, each of 5 min, the water level was dropped to 2 cm above the barrier; also, the animals did not receive any stimulus.

All animals were trained using the same procedure. This consisted of a daily session of 10 trials separated by an intertrial interval (ITI) of varying duration, ranging between 1 and 2 min, for 20 days of training. At the end of the ITI, the trial began: the discriminative stimulus was turned on for a maximum duration of 15 s in the compartment where the fish was located. If the fish did not respond (swimming across the barrier) within 10 s of the onset of the green light, the electric shock was turned on for a maximum of 5 s. Thus, the

temporal separation between cue onset and shock onset was 10 s. A response during the first 10 s finished the warning stimulus (green light) and the shock was not delivered. A response during the period 10–15 s cancelled both the warning stimulus and the shock. The latency defined the type of response in each trial. The responses were classified in two different categories: avoidance responses (shuttle responses occurring before shock onset; the latency was less than 10 s); and escape responses (shuttle responses occurring during the shock; the latency was between 10 and 15 s). Before and after the daily training session, the subject rested in the shuttle box for 5 min without any stimulus presentation.

Spatial task

Apparatus

The experimental apparatus was similar to a previous design (Ingle & Sahagian, 1973; Fig. 2A). It consisted of a square-shaped box made out of grey PVC, with an area of 324 cm^2 and walls 24 cm high. In each of the four corners of this space there was an opening 4 cm wide and 20 cm high. In two of these corners diagonally opposed, there were two cylindrical start areas (20 cm diameter) and the other two doors were used as exits from the experimental apparatus. For each trial only one start area was used while the other was blocked with a grey PVC door (Fig. 2B and C). One of the exits was blocked with translucent glass, leaving the other open as the only goal. Two of the walls of the square-shaped box were covered with panels with five white verticals strips, 2 cm wide, and the other two with panels with nine white circles of 4 cm diameter forming an 'X'.

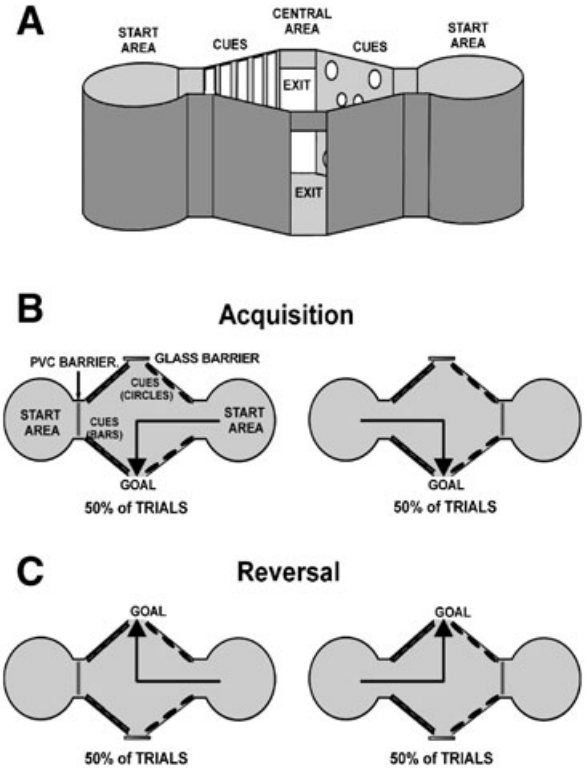

FIG. 2. Schematic representation of the apparatus for spatial learning and spatial task procedure. (A) Scheme of apparatus for spatial training. (B) Procedure of training in the acquisition phase. The position of the goal, internal cues and glass barrier were the same for the whole period of acquisition. The start areas were used in a pseudorandom sequence, so that correct choices required alternation of turns. (C) Procedure of training in the reversal phase. The position of the goal was changed and maintained during the reversal phase. An error was scored when the fish bumped against the glass barrier, and a correct choice was scored when its head passed through the goal.

This structure was placed diagonally in a glass aquarium (1 × 1 m) with an opaque white floor. The aquarium water was aerated and filtered continuously at 22 °C ± 1 to a depth of 22 cm. A grey curtain surrounded this set to make sure that no salient cues were available except those provided by the experimental setting. The experimental box was illuminated with a fluorescent light, placed at a height of 30 cm, orientated along the axis between both start compartments keeping the experimental room in the dark. The fish were trained in groups of four. Two enclosures limited by glass barriers, placed in the aquarium behind the start cylinders, served as resting areas for the subjects during intertrial intervals. The subject within the box could not see the others in the resting area until having passed through the goal, completing the trial.

Spatial procedure

The spatial task of the present study was previously used to test the spatial capabilities of fish (Ingle & Sahagian, 1973) and mammals (Thinus-Blanc & Ingle, 1985). In this task, locating the goal required fish to determine the spatial relationship between cues within the test environment and the goal (López *et al*., 1999). The reversal of the task reveals the type of strategy that fish use. In this sense, fish using a spatial strategy readily transfer the initial learning to the reverse situation (López *et al*., 1999, 2000b). By contrast, a negative transfer is observed in the reversal task when fish use a cue-guidance or a turn strategy.

Before training, the animals were pre-exposed to the apparatus by swimming five times from each start box in random alternation and making spontaneous exits through the two exists of the experimental box. During this phase the glass barrier was not used.

The acquisition phase consisted of 25-trial sessions daily on consecutive days. To begin each trial, the animal was placed carefully on the start compartments (pseudorandom order); with free access to the experimental box (Fig. 2B). If a fish did not make a choice within 30 s it was returned to the start box and gently prodded with a glass rod to motivate an exit. An error was scored when the fish bumped against the glass barrier, and a correct choice was scored when its head passed through the goal before making an error. The relative position of the cues and the correct goal remained constant throughout the experiment for each fish, but the completed maze was rotated 180° four times per session to ensure that the internal panels of experimental box were the only relevant cues. Testing continued at the rate of one session per day until the animals reached the learning criterion (80% correct choices in a session of 25 trials).

When the animals reached the learning criterion the reversal phase began. During reversal training the position of the goal in the experimental box, with respect to the cues, changed, and the animals had to learn the new location of the goal (Fig. 2C). The internal cues and start cylinders remained in the same configuration as in the acquisition phase, and the training procedure was identical to the previous period. This new training concluded when the animals reached the learning criterion (80% correct choices in a session of 25 trials).

Results

During the training an intact animal and another from the LP group died. Another fish from the LP group was discarded because a brain haemorrhage was detected during the histological analysis. In consequence, the number of fish within each group was $n = 8$ (MP); $n = 6$ (LP); $n = 7$ (Control). No statistically significant

differences were observed between the sham and intact animals in either the avoidance responses (Mann–Whitney test: $Us \geq 1$, $Ps > 0.07$), or in the spatial learning (number of sessions to criterion: $U = 3$, $P = 0.18$ and $U = 4.5$, $P = 0.55$, during acquisition and reversal periods, respectively; errors to criterion: $U = 1$, $P = 0.07$ and $U = 3$, $P = 0.28$, during acquisition and reversal periods, respectively) through the 10 blocks of sessions. Therefore data from the sham and intact fish were pooled into a control group ($n = 7$) for statistical analysis.

The extent of the lesions is represented schematically in Figure 3. The histological analysis of injured brains showed that MP lesions affected between 61 and 85% of the ventral region (ventral part of dorsomedial telencephalon; Dmv), and LP lesions affected between 51 and 75% of the ventral region (ventral part of dorsolateral telencephalon; Dlv). The adjacent pallial areas: area dorsalis telencephali pars dorsalis (Dd), area dorsalis telencephali pars centralis (Dc), and the subpallial region area ventralis telencephali pars dorsalis (Vd) were not injured or were minimally affected. The nomenclature used here has been described previously (Peter & Gill, 1975; Nieuwenhuys & Meek, 1990; Portavella *et al*., 2004a,b).

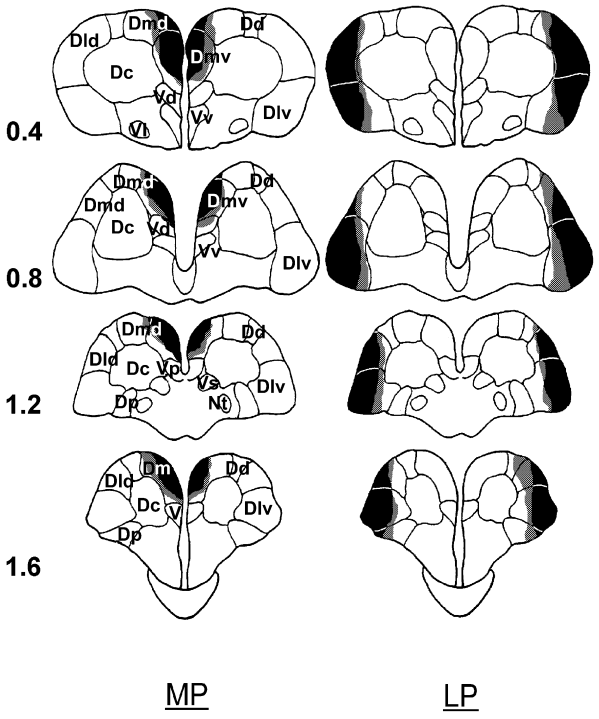

FIG. 3. Graphic representation of the localization and extension of the lesion in the lateral (LP) and medial pallium (MP) in the fish brain. Plates are based on a goldfish brain atlas (Peter & Gill, 1975) and are arranged following the anterior–posterior axis (in millimetres from the anterior pole of telencephalon). Gray shading represents the largest extension and black shading represents the smallest. The nomenclature used in these frontal sections follow that reported elsewhere (Peter & Gill, 1975; Nieuwenhuys & Meek, 1990; Portavella *et al*., 2004a,b). ac, anterior commisure; Nt, nucleus taenia; Pallium: Dc, area dorsalis telencephali pars centralis; Dd, area dorsalis telencephali pars dorsalis; Dld, area dorsalis telencephali pars lateralis; Dlv, area dorsalis telencephali pars lateralis ventralis; Dmd, area dorsalis telencephali pars medialis; Dmv, area dorsalis telencephali pars medialis ventralis. Subpallium: Vd, area ventralis telencephali pars dorsalis; Vl, area ventralis telencephali pars lateralis; Vp, area ventralis telencephali pars postcommisuralis; Vs, area ventralis telencephali pars supracommisuralis; Vv, area ventralis telencephali pars ventralis. The Dmv is considered the ventral part of medial pallium, and it has been proposed homologous to pallial amygdala of land vertebrates. The Dlv is considered the ventral part of the lateral pallium and it has been proposed as being homologous to the hippocampus.

Avoidance learning

The data from the two-way avoidance procedure (Fig. 4A) showed that the control and LP groups reached a high level of avoidance response in the last block of two sessions: 76.42% ± 4.84 (mean ± SEM) and 72.5% ± 2.5, respectively, through 20 experimental sessions. In contrast, the MP group displayed a low level of avoidance responses (37.14% ± 6.97). These differences were statistically significant (ANOVA with repeated measures; $F_{2,18} = 15.233$, $P < 0.001$). There were no differences between the control and LP groups (*post hoc* analyses: DHS-Tukey $\alpha = 0.01$, $P > 0.7$). However, the differences between the MP group and the LP and control groups were statistically significant (DHS-Tukey $\alpha = 0.01$, *P*-values < 0.001).

The analysis of the latency response (Fig. 4B) showed that the values of control and LP groups were less than 10 s (avoidance

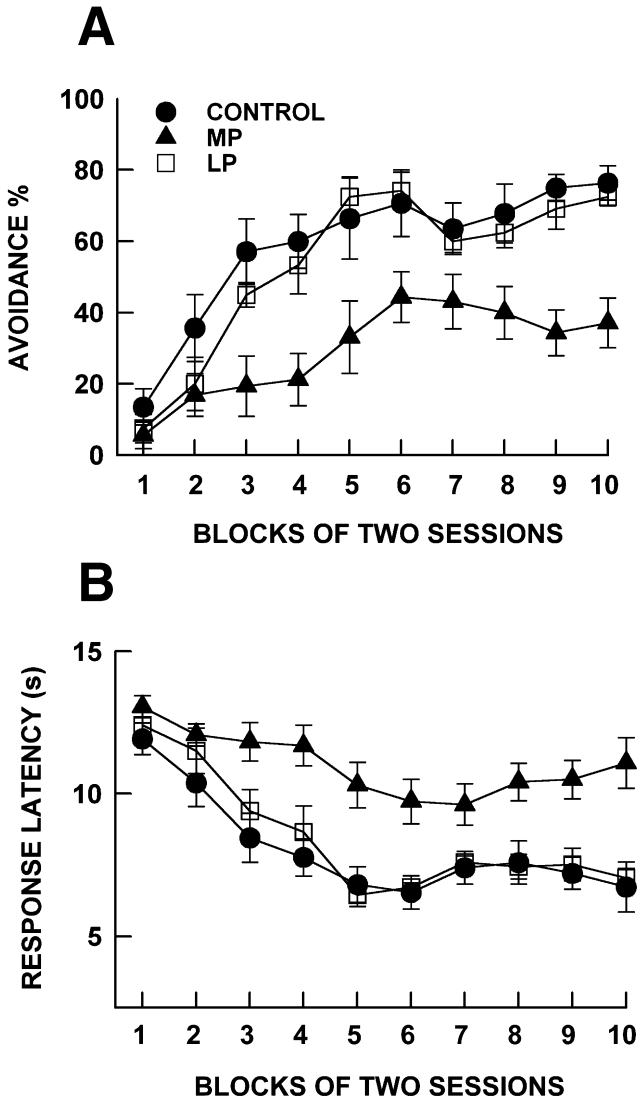

FIG. 4. The medial pallium (MP) but not the lateral pallium (LP) impairs two-way avoidance. (A) Percentage of avoidance responses through 20 days of training. The percentage of avoidance responses of the MP group was significantly lower than the control and LP groups. (B) Response latencies through 20 days of training. The latency of the MP group reached values of escape response, superior to 10 s; in contrast the control and LP group showed latency values of avoidance response, inferior to 10 s. Data was grouped in 10 blocks of two sessions each for analysis and graphical representation [1: 1 & 2 sessions (Ss); 2: 3 & 4 Ss; 3: 5 & 6 Ss; 4: 7 & 8 Ss; 5: 9 & 10 Ss; 6: 11 & 12 Ss; 7: 13 & 14 Ss; 8: 15 & 16 Ss; 9: 17 & 18 Ss; 10: 19 & 20 Ss].

latency) in the last block of two sessions: 6.73 s ± 0.87 and 7.05 s ± 0.34, respectively. In contrast, the latency response of the MP group was higher than 10 s (escape latency 11.08 s ± 0.89). The different latency values of control, LP and MP groups were statistically significant ($F_{2,18} = 14.267$, $P < 0.001$). No differences were found between the control and LP groups (*post hoc* analyses: DHS-Tukey $\alpha = 0.01$, $P > 0.8$). But, the differences were statistically significant between the MP group and the control and LP groups (DHS-Tukey $\alpha = 0.01$, *P*-values < 0.01).

Spatial learning

Acquisition

The number of sessions to reach the criterion was very similar between the control, MP and LP groups: 3.28 ± 0.18; 3.12 ± 0.12; 3.33 ± 0.21, respectively (Fig. 5B). The number of errors to criterion was also similar: 25 ± 1.36 controls; 24.62 ± 1.34 MP; and 27 ± 1.93 LP. There were no significant differences between the three experimental groups (control, LP and MP; Fig. 5A) neither in the number of sessions (ANOVA with one factor; $F_{2,18} = 0.43$, $P > 0.65$) nor in the number of errors ($F_{2,18} = 1.28$, $P > 0.3$) to reach the learning criterion. Therefore, the performance task was the same for all experimental groups (Fig. 5B).

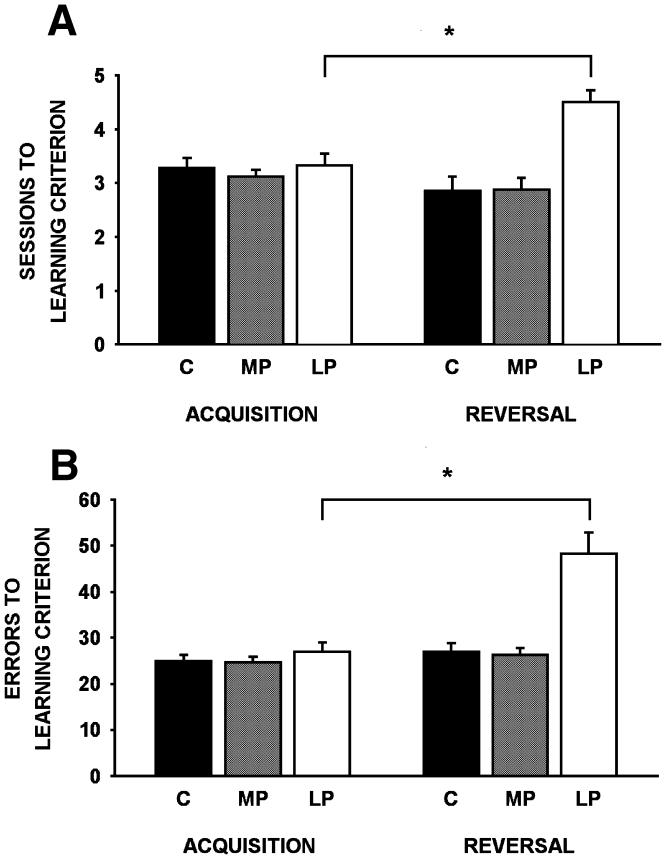

FIG. 5. Lesion to the lateral pallium (LP) but not to the medial pallium (MP) impairs spatial learning. (A) Comparison between three experimental groups in the number of sessions necessary to reach the learning criterion during acquisition and reversal of the spatial task. There were no between-group differences in the number of sessions to learning criterion during the acquisition phase; by contrast, in the reversal phase the LP group needed more sessions to reach the learning criterion. (B) Comparison between the three experimental groups in cumulated errors to learning criterion during acquisition and reversal of the spatial task. There were no differences in the acquisition phase in cumulated errors to criterion between the three groups; by contrast, in the reversal phase the LP group cumulated more errors to reach the learning criterion. C, control group; MP, medial pallium lesion; LP, lateral pallium lesion. *$P < 0.01$.

Reversal

In this case, the three experimental groups ultimately learned the new situation of the goal, but there were significant differences in the number of required sessions ($F_{2,18} = 14.23$, $P < 0.001$) and errors to criterion ($F_{2,18} = 20.56$, $P < 0.001$; Fig. 5). The control and MP groups showed a similar performance both in number of sessions (*post hoc* analyses: DHS-Tukey $\alpha = 0.01$, $P > 0.77$) and in errors to criterion (DHS-Tukey $\alpha = 0.01$, $P > 0.86$). In contrast, the LP group presented statistically significant differences compared with the control and MP groups in number of sessions (DHS-Tukey $\alpha = 0.01$, *P*-values < 0.01) and errors to criterion (DHS-Tukey $\alpha = 0.01$, *P*-values < 0.001). The number of sessions (4.5 ± 0.22) and errors to criterion (48.33 ± 4.54) of the LP group was higher than the control (2.85 ± 0.26 sessions and 27 ± 1.91 errors to criterion) and MP groups (2.87 ± 0.23 sessions and 26.4 ± 1.4 errors).

The comparison between the acquisition and reversal phases (Fig. 2B and C) for each experimental group indicated that the control and MP groups needed less sessions to reach the criterion in the reversal task than in the acquisition period (Fig. 5A), but this difference was not significant (*t*-test for related samples; $t_6 = 2.12$, $P > 0.07$ and $t_7 = 1.53$, $P > 0.17$, respectively). The differences in accumulated errors were not significant either (control group: $t_6 = 1.84$, $P > 0.11$; MP group: $t_7 = 1.25$, $P > 0.29$; Fig. 5B). However, the LP group required more sessions to reach the learning criterion during the reversal of the task ($t_5 = 3.79$, $P = 0.013$; Fig. 5A) and made more errors to reach the criterion than in the acquisition phase ($t_5 = 5.65$, $P = 0.002$; Fig. 5B).

Discussion

The results clearly show that lesions of the medial pallium in goldfish impair the acquisition of an avoidance conditioning response in the same way that the whole telencephalic ablation does (Overmier & Hollis, 1990; Overmier & Papini, 1985, 1986; Papini, 1985; their figure 6). Interestingly, amygdalar lesions produce the same effects in land vertebrates (McIntyre & Stein, 1973; Grossman *et al*., 1974; Werka *et al*., 1978). In contrast, this lesion does not affect the learning of the spatial task (Fig. 5).

Furthermore, the ablation of the lateral pallium produces the opposite effect of the medial pallial lesions. It does not produce either improvement – as described after hippocampal lesions – (Burgess *et al*., 1999; O'Keefe & Nadel, 1978; Eichenbaum *et al*., 1999) or deficit in the acquisition of avoidance conditioned response in comparison to the control group (Fig. 4). In contrast, it produces an important deficit in the spatial learning capability of the fish. In our experiment, this deficit was manifested in the increment of errors and sessions necessary to learn the new goal position in the spatial reversal task (Fig. 5). Thus, this effect is very close to the impairment produced by hippocampal lesions.

A number of authors have proposed that the ventral part of the medial pallium could be homologous to the basal ganglia or the hippocampus of mammals (Echteler & Saidel, 1981; Murakami *et al*., 1983; Ito *et al*., 1986; Parent, 1986). If this hypothesis is correct, the medial pallium could have functional similarities with the mammalian basal ganglia. Nevertheless, the present results demonstrate that a medial pallial lesion does not impair the motor response of any task, as shown by the values

from the spatial learning task and latency from the avoidance task in comparison with the control group (Figs 4 and 5). Therefore, damage to the medial pallium does not produce any effects similar to damage to basal ganglia or the hippocampus suggesting that the functional homology within this structure is unlikely.

Telencephalon-based systems of memory in fish could be based on homologous structures between vertebrate groups

The results indicate that the lateral pallium is involved in spatial information processing. Thus, despite LP lesions not preventing the animals from acquiring the task or from finding the position of the new goal in the reversal of the task, the efficiency of the reversal of the task was reduced significantly compared with the control and MP groups (Fig. 5). This difference could be explained by the existence of at least two different learning systems to solve the spatial task, just as is proposed by the theory of the spatial map (cartographic vs. taxon learning; O'Keefe & Nadel, 1978), or the theory for individual vs. relational learning in mammals (O'Keefe & Nadel, 1978; Burgess *et al*., 1999; Eichenbaum *et al*., 1999). In fact, an essential characteristic from the cartographic and relational model of memory is its flexibility and capacity to detect the changes in the relations between the environmental cues, which facilitate the re-learning of the task. In consequence, the reversal learning will be quicker than acquisition. However, taxon and individual memory can detect simple environmental changes (i.e. presence of a direct cue, or absence of this cue), but do not detect changes in the relation between an environmental cue set. In this case, the memory system must ignore a previously acquired simple spatial representation and learn a new relation, *de novo*, with the same requirements but competing with the earlier representation. In consequence the animal's performance could be less effective in spatial reversal. Accordingly, our results suggest that animals with the LP lesion used a taxon or individual memory system to solve the task because the number of sessions and accumulated errors was larger in the reversal of the task compared to the acquisition period. In contrast, control and MP groups utilized a cartographic or relational learning system as the number of sessions and accumulated errors was similar in both the acquisition and the reversal of the task (see López *et al*., 1999 for a detailed discussion on the topic and spatial maze). Even so, the performance of the animals cannot be attributed either to the detection of the glass barrier, odour or visual detection of the fish in the enclosures, located just outside the test apparatus (used to hold fish during the intertrial intervals) as the performance of all three groups at the beginning of the reversal dropped below chance level, indicating that the animals were not using cues other than the ones provided by the experimental enclosure.

Although animals that received a MP lesion showed a higher latency to shuttle response when compared to controls, they also exhibited shorter escape latencies following shock onset (from 0.5 to 1 s measured from shock onset). These results indicate that the effect observed with the medial pallium lesion cannot be attributed to a sensorimotor impairment (i.e. higher pain threshold). In addition, no differences were observed between latency responses and avoidance percentages in the first four training sessions among the three experimental groups. This is consistent with our previous results showing that pallial lesions do not affect the sensorimotor performance of goldfish in this task (Portavella *et al*., 2003, 2004b). Thus, a disruption on stimuli pairing after medial pallium lesions in fish (e.g. pairing a painful stimulus – electric shock – with a visual stimulus – green light) might be similar to what has been described in mammals following basolateral amygdala lesions.

These specific effects of pallial lesions are in agreement with previous studies (Portavella *et al*., 2004a, b) in which the medial pallium lesion impaired the retention of an avoidance conditioned response (in both nontrace and trace avoidance conditioning) and the acquisition (spaced-trial avoidance learning), in the same way as pallial amygdala lesions in mammals (LeDoux, 1995).

In short, past (Rodríguez *et al*., 2002; Portavella *et al*., 2004a) and present results suggest the existence of different telencephalon-based memory systems in actinopterygian fish (i.e. spatial and emotional) based on functionally specialized areas. Moreover, this work finds new evidence of the involvement of the lateral pallium in spatial, contextual, and/or relational memory system, and also shows that medial pallium plays a role in an emotional memory system in fish. Once again, these results support the homology hypothesis on functional features, between the mammalian hippocampus and pallial amygdala and the fish lateral and medial pallium, respectively.

Implications for the brain evolution of vertebrates

This work does not try to take the place of neurochemical, neuroanotomical or developmental studies, which are the natural source of arguments to establish homologous relationships. However, if the actinopterygian brain undergoes a developmental eversion process (Braford, 1995; Nieuwenhuys, 1963; Northcutt & Braford, 1980; Nieuwenhuys & Meek, 1990; Northcutt, 1995; Butler, 2000; Fig. 1), then, following the parsimony principle, it is possible that the basic functions of the major pallial areas have been preserved in broad outline through evolution (Northcutt, 1995). We think that this proposal can be accepted without denying that brain development has not been the same between species, where, some vertebrate groups can have acquired new functional specializations and lost others, or simply the weight of each one may have changed. In addition, it is probable that the abilities to relate punitive or dangerous events with emotional responses (medial pallium like amygdalar system) and to learn environmental changes efficiently (lateral pallium like hippocampal system) are fundamental to the species' survival. So, these results support that a common ancestor of actinopterygian fish and sarcopterygian fish (group from which land vertebrates evolved) could have had a medial and lateral pallia involved in spatial and emotional learning, 400 million years ago (Carroll, 1988). In other words, these primitive and useful neurobehavioural solutions of vertebrates have been conserved during evolution.

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