

Lesions of the medial pallium, but not of the lateral pallium, disrupt spaced-trial avoidance learning in goldfish (*Carassius auratus*)

Manuel Portavella^{a,*}, Blas Torres^b, Cosme Salas^a, Mauricio R. Papini^c

^aLaboratorio de Psicobiología, Departamento de Psicología Experimental, Universidad de Sevilla, C/ Camilo José Cela s/n, E-41018, Sevilla, Spain

^bLaboratorio de Neurobiología, Departamento de Fisiología y Zoología, Universidad de Sevilla, C/ Profesor García González, s/n, E-41012, Sevilla, Spain

^cDepartment of Psychology, Texas Christian University, Box 298920, Fort Worth, TX 76129, USA

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Abstract

The effects of telencephalic lesions of the medial pallium (MP) and lateral pallium (LP) of goldfish on avoidance learning were studied in a two-way, shuttle response, spaced-trial avoidance conditioning situation. Animals received one trial per day, a training regime that permits the assessment of avoidance learning in the absence of stimulus carry-over effects from prior trials. Control and LP-lesioned goldfish exhibited significantly faster avoidance learning than MP-lesioned animals. These results suggest that the MP, but not the LP, is responsible for the widely described deficits in avoidance learning after lesions of the entire telencephalon. The proposal of a functional similarity between the fish MP and the mammalian amygdala, known to be involved in fear conditioning, suggests a conservative phylogenetic role of this area in avoidance learning.

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Recent results demonstrated that teleost fish are able to learn a conditioned avoidance response in a spaced-trial procedure in which training is administered at a rate of one trial per day [15]. The capacity of fish for avoidance learning under spaced-trial conditions implies that the acquired avoidance response can develop in the absence of stimulus carry-over effects from the discriminative stimulus, the shock, and response-feedback stimuli occurring in previous trials [1]. Such capacity is, therefore, fully dependent on the ability of the discriminative stimulus to associatively reinstate a mediational state of fear, as has been hypothesized to be the case in mammals [5,8,12,13,15,19]. In addition, spaced-trial avoidance learning was found to be impaired by bilateral telencephalic ablations [15], as had been previously described for massed-training avoidance learning with multiple trials per session [12]. In mammals, lesion studies indicate that whereas amygdectomy reliably impairs two-way, massed-training avoidance learning [18], hippocampectomy leads to either no effect or actually improves avoidance performance [6]. The mammalian hippocampus and amygdala have been hypothesized to be

homologous, respectively, to the ventral part of the lateral telencephalic pallium (LP) and medial telencephalic pallium (MP) of actinopterygian fish [2,3,9,10].

Complete telencephalic lesions in the goldfish involve the removal of both LP and MP, together with additional areas. Thus, such lesions do not permit identification of which area is responsible for the behavioral effects of complete telencephalic ablations. Restricted lesions are needed to map the functional differentiation of the fish telencephalon. In one experiment [16], lesions of the MP impaired the acquisition of a conditioned avoidance response trained under massed-trial conditions (ten trials per session with an inter-trial interval averaging 120–240 s). Avoidance deficits after MP lesions were observed whether the discriminative stimulus and shock overlapped or were separated by a 5 s trace interval (in trials without an avoidance response). The same experiment also showed that lesions of the LP affected avoidance learning trained under the trace procedure, but had no effect in the regular overlapping procedure [16]. These results suggest a differential role of the fish MP and LP on avoidance learning. However, the training procedure used in this experiment does not permit a distinction between an effect of MP lesions on fear conditioned to the discriminative stimulus vs. an effect on stimulus carry-over effects that can, under regular circum-

* Corresponding author. Tel.: +34-954-55-4401; fax: +34-954-55-1784.
E-mail address: portavel@us.es (M. Portavella).

stances, acquire control over the avoidance response. The present experiment was designed to determine whether the emotional learning system responsible for the effects of complete telencephalic ablations on spaced-trial avoidance learning [15] is located in the MP or in the LP. Training involved a single trial per day, thus effectively eliminating the control of the avoidance response by stimulus carry-over effects and allowing for a more clear assessment of the role of MP in fear conditioning.

Twenty goldfish, 9–11 cm in body length, were group housed in 200 l glass aquaria with aerated and filtered water, and kept at a constant temperature of 20 °C. The room was on a 14:10 h light/dark cycle (lights on at 8:00 a.m.). Pellets of dry food (Tetra-Pond, Ulrich Baemsch GmbH, Germany) were provided daily ad libitum during the entire course of the experiment.

In preparation for telencephalic lesions, animals were anesthetized by immersion in a solution of tricaine methanesulfonate (MS222, Sigma, USA; 1:20,000 p/v). The MP ($n = 6$) and LP ($n = 6$) were aspirated with a micropipette connected to a manual vacuum system. There were also groups of sham-operated ($n = 4$) and intact ($n = 4$) goldfish. After behavioral testing was completed, the brains from MP, LP, and sham-operated animals were removed and cut in 50 μm thick transversal sections for histological analysis.

All animals received training in a two-way, spaced-trial avoidance task (one trial per day during 30 days). Four similar shuttle tanks (50 $\times$ 14 $\times$ 25 cm) were used. Black PVC covered each long side, the floor by white PVC, and two box ends rested clear and translucent to permit the green light presentation as signal (10 W, 220 V ac 50 Hz) for a maximum of 15 s. On each long side a stainless steel bar attached to a metallic plate was used as an electrode to deliver a uniform and mild electric shock as reinforcer (0.39 V/cm, 50 Hz, pulsed 200 ms on and 800 ms off) for a maximum of 5 s. A trapezoidal barrier separated the shuttle box in two compartments. It was 7.5 cm high, 10 cm wide at the top and 18 cm wide at the bottom. The water level over the barrier was kept constant at 2 cm. This resulted in a level of 9.5 cm in each of the compartments.

Each daily trial started with an interval of variable duration, ranging between 120 and 240 s in length, at the end of which the discriminative stimulus (green light) was turned on for a maximum duration of 40 s. A response (swimming over the barrier to the other compartment) during the initial 20 s of green light presentation terminated the stimulus and cancelled the shock. If the animal did not respond within 20 s of stimulus onset, the reinforcer (a mild electric shock: 0.39 V ac/cm, 50 Hz sinusoidal stimulation) was turned on for a maximum of 20 s. A response during this period terminated both the green light and the shock. If the animal failed to respond altogether, the green light and shock were terminated and an interval of variable duration (ranging between 120 and 240 s) was initiated. The trial ended at the end of this interval; the goldfish was then removed from the conditioning tank and placed back

in its tank. The start side of the shuttle box alternated from day to day, so that the next day the fish were started on the opposite side.

The main dependent variable was the latency to respond, defined as the time since the onset of the green light to either the occurrence of the shuttle response or the termination of the trial, when there was no response. Responses were classified as avoidance responses (shuttle responses occurring before shock onset; the latency was less than 20 s), escape responses (shuttle responses occurring during the shock; the latency was between 20 and 40 s), and responseless trials (trials in which no shuttle response was recorded; the latency was equal to 40 s). The percentages of escape responses and responseless trials were recorded and analyzed to assess the possible emergence of other learning phenomena (e.g. learned helplessness) and to ensure that differences in avoidance responses were not related to a general reduction of activity. The use and handling of the animals were in agreement with the guidelines established by the Directive 86/609/CEE of the European Community Council and the Spanish R.D. 223/1988.

Fig. 1 shows the reconstruction of MP and LP lesions after histological analysis. MP lesions principally affected the ventral part of dorsomedial telencephalon and collaterally dorsocentral and ventral supracommissuralis telencephalon. The LP lesion affected dorsolateral telencephalon (ventral and dorsal parts), and collaterally dorsocentral and dorsoposterior telencephalon. Significant portions of ventral parts of dorsomedial and dorsolateral telencephalon were ablated. The performance of sham-operated ($n = 4$) and intact ($n = 4$) animals exhibited non-significant differences in all the variables analyzed: latency (Mann–Whitney test, $U > 4$, $P > 0.24$, for three blocks of ten trials), avoidance ($U > 4.5$, $P > 0.29$), escape ($U > 6.5$, $P > 0.61$), and responseless ($U > 2$, $P > 0.11$). Therefore, these animals were pooled into a single control group ($n = 8$) for comparison with animals that received MP or LP lesions.

Fig. 2A shows the results of the experiment in terms of the latency measure. A statistical analysis of latencies indicated significant group differences in the third trial block (Kruskal–Wallis test, $\chi^2 = 10.542$, $P < 0.01$). Control and LP groups were not significantly different (Mann–Whitney test, $U = 13$, $P > 0.15$), but the MP group differed significantly from both LP and control groups ($U < 4$, $P < 0.02$). The control and LP groups reached latency values corresponding to avoidance responses (i.e. under 20 s) (Friedman test, $\chi^2 > 10.33$, $P < 0.006$). In contrast, the MP group showed latencies that corresponded to escape responses (i.e. above 20 s) (Friedman test, $\chi^2 = 2.33$, $P > 0.31$).

Fig. 2B displays the results in terms of percentage avoidance responses. Statistically significant group differences were observed in the third trial block ($\chi^2 = 8.92$, $P < 0.012$). No differences were found between sham and control groups ($U = 17$, $P > 0.35$) that reached progressively 73–76% of avoidance responses ($\chi^2 > 7.58$, $P < 0.023$). In contrast, the MP group was significantly

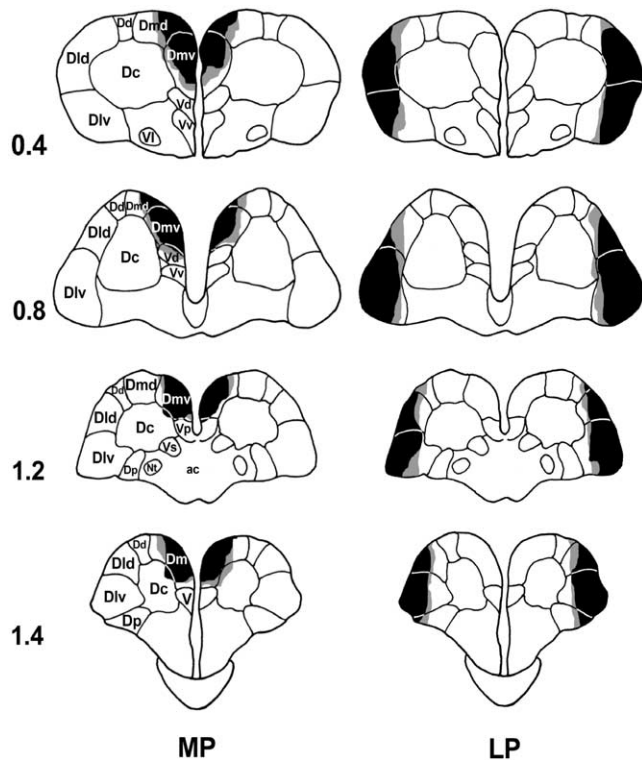


Fig. 1. Schematic representation of the extension of the lesions in LP and MP in the brain fish. Gray shading represents the largest extension and black shading the smallest. These frontal sections have been reconstructed based on the goldfish brain atlas of Peter and Gill [14]. ac, anterior commissure; 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 postcommissuralis; Vs, area ventralis telencephali pars supra commissuralis; Vv, area ventralis telencephali pars ventralis. Dmv is considered the ventral part of MP, and it has been proposed as homologous to pallial amygdala of land vertebrates. Dlv is considered the ventral part of LP and it has been proposed as homologous to hippocampus [4,6,11,12].

different from both control and LP groups ($U < 4.5$, $P < 0.012$). MP animals maintained a low, constant level of avoidance response of about 41% ($\chi^2 = 3.08$, $P < 0.21$). These results showed that whereas MP lesions impaired avoidance learning, LP lesions had no detectable effect on the acquisition of the avoidance response.

Fig. 2C,D show the results in terms of the percentage of trials with escape responses and responseless, respectively, and Table 1 summarizes the statistical results. The progressive decline of control and LP groups in escape performance is correlated with the development of a high level of avoidance behavior ($\chi^2 > 9.18$, $P < 0.011$). In contrast, the MP group showed a high, stable level of escape responses, above 50% ($\chi^2 = 1.75$, $P > 0.41$). The analysis of responseless revealed no behavioral or motor interference on the performance of any of the groups. In summary, both LP and control groups learned the avoidance response at

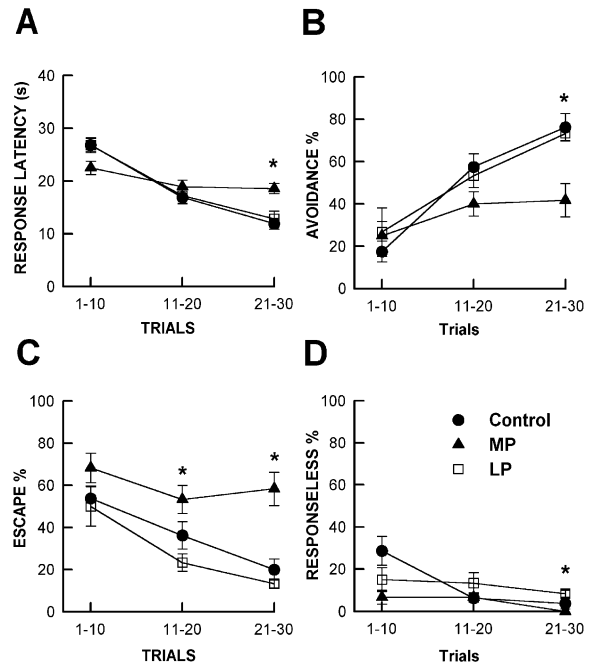


Fig. 2. Results from the experiment. Data are grouped in blocks of ten trials training (one trial training per day). (A) Means of shuttle response latencies $\pm$ SEM. (B) Means of avoidance response percentage $\pm$ SEM. (C) Means of escape response percentage $\pm$ SEM. (D) Means of responseless percentage (absence of shuttle response) $\pm$ SEM. All variables were grouped in sets of ten daily trials (Kruskal–Wallis test, $*P < 0.02$).

about the same rate, while the MP group exhibited no clear evidence of avoidance learning.

The present results show that the MP is involved in spaced-trial avoidance learning. As was mentioned above, the ventral part of MP (area telencephali dorsalis pars medialis ventralis) has been proposed as a homologue of the mammalian amygdala (see references above). The deficit caused by MP damage is similar to deficits in avoidance learning caused by lesions of the amygdala in mammals [17]. Because the present experiment was based on a spaced-trial training procedure, the effects of MP lesions cannot be explained in terms of a disruption of sensory carry-over effects [1,14]. On the contrary, these results support the hypothesis that the MP is involved in the ability of the discriminative stimulus to activate a mediational state of fear whose response-contingent termination maintains avoidance behavior [8,12]. Thus, it is possible to postulate that avoidance learning in fish involves an underlying process of emotional learning.

LP lesions did not produce harmful effects on spaced-trial avoidance learning. It is known that LP lesions affect spatial learning yielding effects that are similar to those obtained with hippocampal lesions in mammals [17]. If the homology between the mammalian hippocampus and the fish LP (area dorsalis telencephali pars lateralis ventralis) is accepted, then available evidence suggests that the function of LP and hippocampus has been preserved through vertebrate evolution. Thus, LP is involved in spatial learning and in avoidance trace conditioning (Portavella, Torres and

Table 1
Statistical analyses of percentage trials with escape responses and responseless

Response	Trials 1–10	Trials 11–20	Trials 21–30
(1) All groups compared (Kruskal–Wallis test)			
Escape	$\chi^2 = 2.3, P > 0.25$	$\chi^2 = 7.6, P < 0.03$	$\chi^2 = 11.4, P < 0.01$
Responseless	$\chi^2 = 5.8, P > 0.06$	$\chi^2 = 1.7, P > 0.42$	$\chi^2 = 7.9, P < 0.02$
(2) Control vs. LP (Mann–Whitney test)			
Escape	–	$U = 13, P > 0.14$	$U = 20, P > 0.54$
Responseless	–	–	$U = 12.5, P > 0.09$
(3) Control vs. MP (Mann–Whitney test)			
Escape	–	$U = 11.5, P > 0.10$	$U = 3.5, P < 0.01$
Responseless	–	–	$U = 18, P > 0.20$
(4) LP vs. MP (Mann–Whitney test)			
Escape	–	$U = 2, P < 0.01$	$U = 0, P < 0.01$
Responseless	–	–	$U = 3, P < 0.01$

Salas, in preparation) [16], but it does not play a detectable role in spaced-trial avoidance conditioning in the absence of a temporal gap between the discriminative stimulus and the aversive reinforcer. All together, these results indicate that the fish LP is involved in relational learning and the processing of the temporal attributes of the training situation, as it has been argued for hippocampal function in mammals [4,7,11].

In conclusion, the results of this experiment show that the behavioral nature of fish avoidance learning is similar to that of mammalian avoidance learning. More specifically, the fish MP plays a role in avoidance learning that is similar to that of the mammalian amygdala. Together with other studies involving trace procedures and spatial learning [16, 17], the present results add to the view that the fish MP and LP areas, which have been proposed as morphological homologues of the amygdala and hippocampus, respectively, also share functional similarities with these mammalian structures. These results provide evidence consistent with a striking degree of evolutionary conservation in both morphology and function of vertebrate pallial systems.

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