

# Chlorpheniramine facilitates inhibitory avoidance in teleosts submitted to telencephalic ablation

F.R. Faganello and R. Mattioli

Laboratório de Neurociência, Departamento de Fisioterapia, Universidade Federal de São Carlos, São Carlos, SP, Brasil

Correspondence to: F.R. Faganello, Departamento de Fisioterapia, Universidade Federal de São Carlos, Via Washington Luis, km 235, 13565-905 São Carlos, SP, Brasil  
Fax: +55-16-3361-2081. E-mail: frfaganello@yahoo.com.br

The present study investigated the involvement of H(1) histaminergic receptor on the acquisition of inhibitory avoidance in *Carassius auratus* submitted to telencephalic ablation. The fish were submitted to telencephalic ablation 5 days before the experiment. The inhibitory avoidance procedure included 1 day for habituation, 3 days for training composed of 3 trials each (1st day: T1, T2, T3; 2nd day: 2T1, 2T2, 2T3; 3rd day: 3T1, 3T2, 3T3) and 1 day for test. On training days, the fish were placed in a white compartment, after 30 s the door was opened. When the fish crossed to a black compartment, a weight was dropped (aversive stimuli). Immediately after the third trial, on training days, the fish received, intraperitoneally, one of the pharmacological treatments (saline (N = 20), 8 (N = 12) or 16 (N = 13)  $\mu\text{g/g}$  chlorpheniramine, CPA). On the test day, the time to cross to the black compartment was determined. The latency of the saline group increased significantly only on the 3rd trial of the 2nd training day (mean  $\pm$  SEM, T1 (50.40  $\pm$  11.69), 2T3 (226.05  $\pm$  25.01); ANOVA: P = 0.0249, Dunn test: P < 0.05). The group that received 8  $\mu\text{g/g}$  CPA showed increased latencies from the 2nd training day until the test day (T1 (53.08  $\pm$  17.17), 2T2 (197.75  $\pm$  35.02), test (220.08  $\pm$  30.98); ANOVA: P = 0.0022, Dunn test: P < 0.05). These results indicate that CPA had a facilitating effect on memory. We suggest that the fish submitted to telencephalic ablation were able to learn due to the local circuits of the mesencephalon and/or diencephalon and that CPA interferes in these circuits, probably due an anxiolytic-like effect.

Key words: Chlorpheniramine; Inhibitory avoidance; Telencephalic ablation; Teleost

Research supported by CAPES and CNPq. Publication supported by FAPESP.

Received October 2, 2007. Accepted April 17, 2008

The limbic system is critically involved in fear-related classical conditioning in mammals (1). However, ablation of the teleost telencephalon, which is phylogenetically related to the limbic system in land vertebrates, does not disrupt classical autonomic conditioning when using an aversive procedure similar to that used in mammals (1,2), indicating that mesencephalic structures are able to support the learning of conditioned emotional responses.

Teleosts are suitable for studying neurotransmitter histamine in the central nervous system because the histaminergic system in fish has only one area of projection to the telencephalon (3), which has been well preserved through evolution (4).

Previous studies showed that histamine has inhibitory effects on different tasks of inhibitory avoidance. Eidi et al. (5) suggested that post-training administration of histamine attenuated memory retention and potentiated the inhibitory effect on memory induced by scopolamine in rats. An increase in cerebral histamine levels was reported to impair the acquisition of avoidance response, whereas reduced levels facilitated this acquisition (6). In teleosts, chlorpheniramine (CPA), an H1 histaminergic antagonist, improved the learning of inhibitory avoidance (7).

Since histamine causes an inhibitory effect on learning tasks and ablation of the teleost telencephalon does not disrupt classical autonomic conditioning using an aversive

procedure (1,2), the aim of this study was to determine the role of CPA in the consolidation of inhibitory avoidance in teleosts submitted to telencephalon ablation.

Goldfish (*Carassius auratus*) of undetermined sex, weighing 4-10 g, were maintained at 20-25°C in a continuously filtered and aerated 30-L aquarium, 15 fish per aquarium, under a natural light cycle.

The fish were fed five times a week with flake food (Wardly Corp., New Jersey, USA). There was a 2-week acclimation period between the purchase of the fish and the surgical procedure. The fish were identified individually by unique physical characteristics, such as color and tail type.

The fish were anesthetized by immersion in a solution of 0.8 g/L tricaine methanesulfonate (3-aminobenzoic acid ethyl ester methasulfonate; Sigma Chemical Co., USA) until spontaneous motor activity and gill movement ceased. The fish were then wrapped in wet gauze and placed on a stand that stabilized the body by laterally adjustable holders. Anesthesia was maintained by continuously perfusing the animals through the mouth with an aerated solution of 0.3 g/L tricaine methanesulfonate.

The dorsal part of the skull was removed to expose the telencephalic lobes. The telencephalic lobes were then ablated by aspiration through a glass pipette connected to a vacuum pump. Following telencephalic ablation, the skull was closed with dental acrylic (Dental Vipi Ltda., Pirassununga, SP, Brazil). After the skull was closed, the anesthetic solution was replaced by fresh water until the gills began to move. The fish were then placed in the maintenance aquarium for 5 days of recovery prior to the experimental procedure. Survival was 80%.

At the conclusion of the experiment, the fish were decapitated and the brains were removed from the skull and inspected for preliminary assessment of ablation (Figure 1). The brains were then fixed in 10% formalin for 4 days. Thereafter, 50- $\mu$ m sections were prepared with a freezing microtome. The telencephalon-ablated brains exhibited complete telencephalic tissue removal and the preoptic areas and optic tracts had not been injured.

Chlorpheniramine maleate salt (Sigma) dissolved in 0.9% saline to a concentration of 8 and 16  $\mu$ g/ $\mu$ L CPA or 0.9% saline were administered intraperitoneally with a Hamilton syringe (50  $\mu$ L; Hamilton Bonaduz AG, Switzerland) at a volume of 1  $\mu$ L/g. The substances were coded and the experimenter was unaware of which substance was injected into each fish.

The fish were divided into three groups: saline group (SAL, N = 20), 8  $\mu$ g/ $\mu$ L CPA group (CPA8, N = 12), and 16  $\mu$ g/ $\mu$ L CPA group (CPA16, N = 13).

A rectangular aquarium divided into two compartments was used. Each compartment was 15 cm high, 15 cm wide, and 25 cm long. One compartment was black and the other white. A central sliding door was used to separate the compartments.

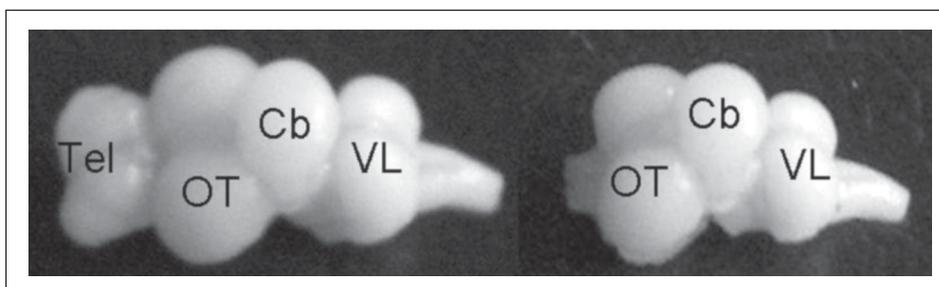
The experiment was performed on 5 consecutive days. One day for habituation, 3 days for training (three trials/day: 1st training, T1, T2 and T3; 2nd training, 2T1, 2T2 and 2T3, and 3rd training, 3T1, 3T2 and 3T3) and 1 day for test.

On the habituation day, each fish was placed in the aquarium by itself for 10 min. On training days, the fish were placed individually in the white compartment and after 30 s the door was opened and the time spent for crossing into the black compartment was recorded (latency). When the fish crossed to the black compartment, a 45-g weight (aversive stimulus) was dropped in front of it (7,8). Thereafter, the fish crossed back or was immediately returned to the start compartment and the procedure was repeated twice. Crossing was defined as the moment when the dorsal fin entered the black compartment. Immediately after the third trial on each of the 3 days of training, the fish received the pharmacological treatments (SAL, CPA8, or CPA16).

Twenty-four hours later the fish were tested. They were placed individually in the white compartment and after 20 s the door was opened and the time before entering the black compartment was recorded again.

The white compartment was chosen as the starting compartment because a previous experiment indicated that goldfish present a natural preference for dark environ-

**Figure 1.** Upper view of a normal brain (left) and of another with a telencephalic ablation (right). Tel = telencephalon; OT = optic tectum; Cb = cerebellum; VL = vagal lobe.



ments (9). All experiments were videotaped.

All results were initially submitted to the Levene test for homogeneity of variance. When appropriate, the data were converted to  $\log_{10}$  and ANOVA was performed, followed by the Dunn multiple comparisons test. Comparison of T1 on the 1st day of training for each of the different groups and on the test day of the groups was carried out using the Kruskal-Wallis test.  $P < 0.05$  was established as the significance level.

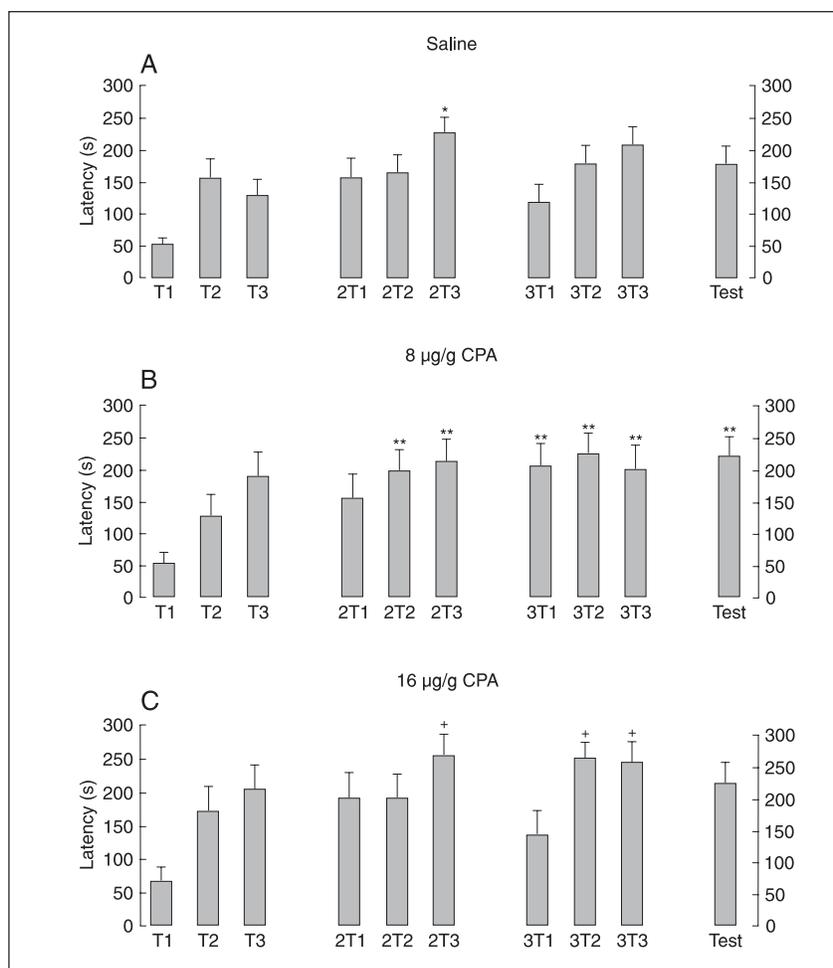
The means  $\pm$  SEM of latencies in seconds for training and test days for the saline-treated group are reported in Figure 2A. The latency was significantly higher only on the 3rd trial of the second training day: T1 ( $50.40 \pm 11.69$ ), 2T3 ( $226.05 \pm 25.01$ ); ANOVA: d.f. = 9,  $F = 2.19$ ,  $P = 0.0249$ , Dunn test:  $P < 0.05$ ).

The fish treated with CPA8 showed increased latencies on trial after trial on the 2nd training day and latencies

remained high for the three trials on 3rd training day and test: T1 ( $53.08 \pm 17.17$ ), 2T2 ( $197.75 \pm 35.02$ ), 2T3 ( $211.75 \pm 36.32$ ), 3T1 ( $205.25 \pm 37.18$ ), 3T3 ( $199.58 \pm 38.67$ ), test ( $220.08 \pm 30.98$ ); ANOVA: d.f. = 9,  $F = 3.15$ ,  $P = 0.0022$ , Dunn test:  $P < 0.05$  (Figure 2B). The CPA16 group showed higher latencies on the 2nd and 3rd days of training T1 ( $66.769 \pm 20.431$ ), 3T2 ( $249.38 \pm 22.486$ ), 3T3 ( $242.85 \pm 30.317$ ); ANOVA: d.f. = 9,  $F = 3.67$ ,  $P = 0.0005$ , Dunn test:  $P < 0.05$  (Figure 2C).

There were no differences among the SAL, CPA8 and CPA16 groups at T1 and on the test day (T1: Kruskal-Wallis: d.f. = 2,  $P = 0.93$ ; test day: Kruskal-Wallis: d.f. = 2,  $P = 0.77$ ).

Inhibitory avoidance is characterized by the reduction of natural or reinforced behavior by the animal incited by an aversive experience (10). Previous studies suggested an innate preference of teleost fish for dark environments (9).



**Figure 2.** Effect of chlorpheniramine (CPA) on inhibitory avoidance in goldfish submitted to telencephalic ablation. Data are reported as means  $\pm$  SEM of latencies in seconds for training and test days. T1, T2 and T3 correspond to three trials of the 1st training day. 2T1, 2T2 and 2T3 correspond to trials of the 2nd training day and 3T1, 3T2 and 3T3 correspond to trials of the 3rd training day. \*Saline group statistically different from T1 (N = 20; ANOVA:  $P = 0.0249$ , Dunn test:  $P < 0.05$ ). \*\*CPA 8  $\mu\text{g/g}$  statistically different from T1 (N = 12; ANOVA:  $P = 0.0022$ , Dunn test:  $P < 0.05$ ). +CPA 16  $\mu\text{g/g}$  statistically different from T1 (N = 13; ANOVA:  $P = 0.0005$ , Dunn test:  $P < 0.05$ ).

In studies in which the same model of inhibitory avoidance task was used, fish took longer to leave the white compartment of the aquarium after experiencing aversive stimulus immediately after crossing to the dark compartment (7,8,11). These data suggest both the effectiveness of the model proposed and the adequacy of the aversive stimulus used. Moreover, we have published data on unoperated groups that indicated that the inhibitory avoidance model used in the present study affects learning and memory and has also been sensitive to histaminergic drugs (7,8).

The results obtained for the saline-treated group suggest that fish were able to learn the task but did not retain the learned information. Treatment with CPA, mainly at 8 µg/g, had a facilitating effect on memory.

The results of the present study suggest that CPA improves memory of the inhibitory avoidance task in telencephalic-ablated fish. Albeit, there were no differences between groups at the beginning and the end of the experimental procedure, CPA-8 group retained performance on the 3rd day. Cofiel and Mattioli (7) found similar results in a study performed on intact goldfish with the same experimental model in which the group treated with 8 mg/kg CPA presented a significant increase in latency before leaving the start compartment. The present study confirms and extends the action of CPA on conditioning of inhibitory avoidance by suggesting that these effects occur via mesencephalic and/or diencephalic structures, as the action of CPA was the same in animals submitted or not to telencephalic ablation.

Blanchard et al. (12) and Fanselow (13) proposed that defensive behavior patterns are organized in a hierarchical series of responses. Some studies suggest that stimulation of the midbrain tectum structures produces alertness, followed by freezing and escape reactions. Furthermore, electrical stimulation of the dorsal periaqueductal gray and inferior colliculus has consistently been shown to induce aversive effects, because it elicits defensive behaviors. Moreover, electrical stimulation sustained learned operant escape responses and also supported learning of conditioned emotional responses (14).

Although the midbrain tectum seems to have local circuits for the generation and elaboration of defense reac-

tions, higher brain structures are necessarily enlisted in the control of more complex fear-related behaviors. For example, the connections of the inferior colliculus to the amygdala act as an important filter for sensorial information of an aversive nature (15). Based on these data, we suggest that the fish submitted to telencephalic ablation were able to learn the inhibitory avoidance task due to the local circuits of the midbrain, and that CPA facilitated retention of an aversive experience, as a type of defensive behavior (16), for at least 24 h.

There is evidence that avoidance learning is based on the acquisition of a mediational state of fear in goldfish (17). In agreement with Brandão et al. (18), the preparatory process of danger-orientation, fear and avoidance seems to be linked to anxiety. Thus, the facilitatory effect of CPA on memory reported here can also be due to an anxiolytic-like effect of CPA mediated by the diencephalon and/or the midbrain tectum.

The anxiolytic-like effect of CPA mediated by the mesencephalon and/or diencephalon in goldfish has been suggested in other studies. In a study by Faganello and Mattioli (11), CPA produced effects similar to those of diazepam, a classic anxiolytic drug, in goldfish submitted to telencephalic ablation in the same model of inhibitory avoidance used in this study. In a study carried out by Medalha et al. (19), the CPA-treated group had lower 5-hydroxyindoleacetic acid levels in the diencephalon than the saline groups, suggesting that CPA may have an anxiolytic-like effect because serotonin is involved in the anxiety process (20).

We suggest that fish submitted to telencephalic ablation were able to learn the inhibitory avoidance task due to the local circuits of the midbrain that act to generate and elaborate defense reactions, and that CPA, probably due to its anxiolytic-like effect, interferes in mesencephalon and/or diencephalon circuits.

## Acknowledgments

We thank Ms. Tereza Fatori, Universidade Federal de São Carlos, São Carlos, SP, Brazil, for technical assistance.

## References

1. Medina JF, Christopher Repa J, Mauk MD, LeDoux JE. Parallels between cerebellum- and amygdala-dependent conditioning. *Nat Rev Neurosci* 2002; 3: 122-131.
2. Overmier JB, Curnow PF. Classical conditioning, pseudoconditioning, and sensitization in "normal" and forebrainless goldfish. *J Comp Physiol Psychol* 1969; 68: 193-198.
3. Ekstrom P, Holmqvist BI, Panula P. Histamine-immunoreactive neurons in the brain of the teleost *Gasterosteus aculeatus* L. Correlation with hypothalamic tyrosine hydroxylase- and serotonin-immunoreactive neurons. *J Chem*

- Neuroanat* 1995; 8: 75-85.
4. Peitsaro N, Kaslin J, Anichtchik OV, Panula P. Modulation of the histaminergic system and behaviour by alpha-fluoromethylhistidine in zebrafish. *J Neurochem* 2003; 86: 432-441.
  5. Eidi M, Zarrindast MR, Eidi A, Oryan S, Parivar K. Effects of histamine and cholinergic systems on memory retention of passive avoidance learning in rats. *Eur J Pharmacol* 2003; 465: 91-96.
  6. Rubio S, Begega A, Santin LJ, Miranda R, Arias JL. Effects of histamine precursor and (R)-alpha-methylhistamine on the avoidance response in rats. *Behav Brain Res* 2001; 124: 177-181.
  7. Cofiel LP, Mattioli R. Involvement of histamine receptors in the acquisition of inhibitory avoidance in *Carassius auratus*. *Prog Neuropsychopharmacol Biol Psychiatry* 2006; 30: 1246-1250.
  8. Faganello FR, Medalha CC, Mattioli R. Haloperidol and chlorpheniramine interaction in inhibitory avoidance in goldfish. *Behav Brain Res* 2003; 147: 83-88.
  9. Serra EL, Medalha CC, Mattioli R. Natural preference of zebrafish (*Danio rerio*) for a dark environment. *Braz J Med Biol Res* 1999; 32: 1551-1553.
  10. Millenson JR, Hendry DP. Quantification of response suppression in conditioned anxiety training. *Can J Psychol* 1967; 21: 242-252.
  11. Faganello FR, Mattioli R. Anxiolytic-like effect of chlorpheniramine in inhibitory avoidance in goldfish submitted to telencephalic ablation. *Prog Neuropsychopharmacol Biol Psychiatry* 2007; 31: 269-274.
  12. Blanchard RJ, Blanchard DC, Hori K. An ethoexperimental approach to the study of defense. In: Blanchard RJ, Brain PF, Blanchard DC, Parmigiani S (Editors), *Ethoexperimental approaches to the study of behavior*. Dordrecht: Kluwer Academic Publishing; 1989. p 114-136.
  13. Fanselow MS. The midbrain periaqueductal gray as a coordinator of action in response to fear and anxiety. In: DePaulis A, Bandler R (Editors), *The midbrain periaqueductal grey matter: Functional, anatomical and immunohistochemical organization*. New York: Plenum Press; 1991. p 151-173.
  14. Brandão ML, Troncoso AC, Melo LL, Sandner G. Active avoidance learning using brain stimulation applied to the inferior colliculus as negative reinforcement in rats: evidence for latent inhibition. *Neuropsychobiology* 1997; 35: 30-35.
  15. Maisonnette SS, Kawasaki MC, Coimbra NC, Brandão ML. Effects of lesions of amygdaloid nuclei and substantia nigra on aversive responses induced by electrical stimulation of the inferior colliculus. *Brain Res Bull* 1996; 40: 93-98.
  16. Misslin R. The defense system of fear: behavior and neurocircuitry. *Neurophysiol Clin* 2003; 33: 55-66.
  17. Portavella M, Salas C, Vargas JP, Papini MR. Involvement of the telencephalon in spaced-trial avoidance learning in the goldfish (*Carassius auratus*). *Physiol Behav* 2003; 80: 49-56.
  18. Brandão ML, Troncoso AC, de Souza Silva MA, Huston JP. The relevance of neuronal substrates of defense in the midbrain tectum to anxiety and stress: empirical and conceptual considerations. *Eur J Pharmacol* 2003; 463: 225-233.
  19. Medalha CC, Santangelo EM, Mattioli R. Neurochemical analysis of brain monoamines after L-histidine and chlorpheniramine administration in goldfish. *Neurosci Lett* 2003; 349: 179-182.
  20. Graeff FG. On serotonin and experimental anxiety. *Psychopharmacology* 2002; 163: 467-476.