

## Effect of telencephalic ablation on appetitive instrumental learning in the goldfish (*Carassius auratus*)

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Goldfish received either bilateral ablation of the telencephalon or a sham operation, followed by instrumental training under widely-spaced conditions (one trial per day). Animals were trained to swim in a runway for either continuous reinforcement or 50% partial reinforcement. Thirty-two daily acquisition sessions were followed by 32 daily extinction sessions during which food was withheld. Telencephalic lesions disrupted early acquisition performance, but lesioned animals achieved an asymptotic performance similar to that of sham controls. Extinction was facilitated by the lesions, independently of the schedule of reinforcement used during acquisition. The results are interpreted in relation to comparative research on spaced-trial appetitive instrumental learning in vertebrates.

*Efectos de la ablación telencefálica sobre el aprendizaje apetitivo instrumental en la carpa dorada (*Carassius auratus*).* En este experimento se emplearon carpas doradas (*Carassius auratus*) con ablación bilateral del telencéfalo o con operación de control en un procedimiento de entrenamiento instrumental con distribución de ensayos ampliamente espaciados (un ensayo por día). Los animales fueron entrenados a nadar en un corredor bajo condiciones de reforzamiento continuo o reforzamiento parcial al 50%. Se realizaron 32 sesiones diarias de adquisición seguidas de 32 sesiones diarias de extinción en las que la comida fue suprimida. La lesión telencefálica afectó a la adquisición durante los primeros ensayos aunque, al final, los animales lesionados alcanzaron una ejecución asintótica similar a la de los animales controles. La extinción se vio facilitada por las lesiones, independientemente del programa de reforzamiento usado durante la adquisición. Los resultados se interpretan en relación a los estudios comparativos de aprendizaje instrumental apetitivo con ensayos espaciados realizados en otros vertebrados.

Experimental lesions of the teleost telencephalon impair associative learning in a variety of training situations. In the goldfish (*Carassius auratus*), for example, bilateral ablations disrupt the acquisition of appetitive visual conditional discriminations (Onishi, 1997), complex spatial learning (Salas et al., 1996), the acquisition of avoidance behavior (Heinsworth et al., 1967), and the retention of preoperatively trained avoidance behavior (Overmier and Papini, 1986). Interestingly, bilateral lesions of the telencephalon do not lead to measurable disruption of learning in relatively simpler situations, such as brightness discriminations (Onishi, 1997), escape conditioning (Heinsworth et al., 1967), and aversive Pavlovian conditioning (Overmier and Curnow, 1969).

Research on learning in animals suggests that often variables that have little or no effects on the acquisition of new behaviors in simple situations, lead to significant effects when animals are shifted to extinction. This is particularly noticeable when practice is administered at a rate of a single trial per day. For example, lesions of the hippocampus in rats (Jarrad et al., 1964) or of the medial pa-

llium (the amphibian homologue) in toads (Muzio et al., 1993; Papini et al., 1995) have no consequences for the acquisition of simple running responses reinforced in all trials (i.e., continuous reinforcement, CR). However, lesioned animals from both species show abnormally high levels of persistence during the extinction of instrumental behavior. Interestingly, the same lesion has opposing effects on extinction when the instrumental behavior was acquired under partial reinforcement (PR). In rats, the hippocampal lesion decreases resistance to extinction (Jarrad et al., 1964; Jarrad and Isaacson, 1965; Winocur and Mills, 1969), whereas in toads, lesions of the medial pallium increase resistance to extinction (Muzio et al., 1993).

An interesting corollary of this pattern of results is that it suggests that PR training engages different brain processes in rats and toads. Hence, the lesion manipulation has opposing effects on extinction after CR vs. PR training in rats (increased persistence after CR, but decreased persistence after PR), whereas an equivalent lesion manipulation has the same effect on extinction in toads (persistence increased after both CR and PR). Such differences in the effects of physiological manipulations are consistent with the hypothesis, suggested by purely behavioral experiments, that CR and PR training engage different learning processes in mammals, but the same underlying learning process in other vertebrates (Papini et al., 1995; Papini, 1997). One candidate mediational process in mammalian learning is provided by the hypothesis that partial

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reinforcement induces the activation of an emotional reaction of frustration elicited by the surprising omission of an appetitive reinforcer (Gray, 1987; Amsel, 1992; Papini and Dudley, 1997). The present experiment was designed to determine if a global lesion of the fish telencephalon affects the extinction of widely-spaced instrumental learning in the same direction (as in amphibians), or in opposite direction (as in mammals), as a function of whether acquisition involved CR or PR.

#### Method

##### *Subjects*

The subjects were 32 experimentally naive goldfish (*Carassius auratus*) purchased from a local aquarium in Seville. Goldfish were 12–15 cm in body length and lived in pairs in tanks measuring 42 x 26 x 15 cm. Tanks were cleaned every 2 days. Water was kept at a constant temperature of 21°C and continuously aerated. The aquarium room was kept under a 14:10 hr light: dark cycle (light from 07:00 hr to 21:00 hr). Extensive handling experience was administered during the 60 days prior to the initiation of the training sessions. Goldfish were individually handled on a daily basis. Beginning 3 weeks before the experiment, a daily feeding routine was introduced. Each goldfish was given 3 pellets of Tetra-pond (Ulrich Baensch GmbH, Germany) fish food (a total of about 0.08 g of food per day).

##### *Apparatus*

Animals received training in a conventional runway made out of white acrylic plates. The runway was 150 cm long X 10 cm wide X 15 cm high, and was divided into three sections. The start and goal boxes were 25 cm long. Manually-operated guillotine doors separated the start and goal boxes from the main section of the runway. A tube measuring 8-mm in diameter was located in the goal box and used to manually deliver the food on reinforced trials. The runway was filled with water up to a level of 12 cm. Water was constantly circulating from the start to the goal box. Opening of the start door activated a digital counter (incrementing in 0.01 s units) that was stopped when the animal activated a photocell located inside the goal box. The latency to traverse the runway was the primary dependent measure.

##### *Surgical and Histological Procedures*

Animals were anesthetized by immersion in a solution of tricaine methanosulfonate (1:20,000). When movement ended, the animal was placed in the surgical chamber where it remained partially bathed in water at a constant concentration of anesthetic (1:20,000). The animal was fixed into position by lateral holders that maintained the body still during surgery. An adjustable tube was inserted into the animal's mouth and connected to a pump that provided a constant flow of water. The concentration of anesthesia was kept constant during surgery.

The dorsal skin and skull were carefully removed and the underlying fatty tissue removed by aspiration. The exposed telencephalon was aspirated with a micropipette connected to a manual vacuum system. Surgery was performed under visual inspection by means of a binocular microscope. Following the ablation, the piece of skull was replaced in its original position, fixed with cyano-

acrylate glue, and covered with dental cement. The fish was then returned to its home tank for a recovery period of 6 days. Sham operations were performed exactly as described, except that the nervous tissue was not injured.

At the end of the experiment, fish were deeply anesthetized (1:5,000) and perfused with 50 ml of 0.9% saline solution, followed by 125 ml of fixative solution (10% formalin in phosphate buffer 0.1 M, pH 7.4). The brains were removed from the skull, inspected for a preliminary evaluation of the ablation, and cut in 50-mm thick transversal sections for histological analysis.

##### *Training Procedure*

All fish received 7 daily sessions of pretraining immediately after the 6 days of post-surgical recovery. During the initial 5 sessions, each animal was exposed to the runway apparatus with the doors raised and without any food for a total of 5 min per session. During sessions 6 and 7, each goldfish was confined for 5 min to the goal box of the runway where it received a total of 6 pellets of Tetra-pond fish food (approximately 0.15 g).

Training began on day 8. Sham-operated and ablated animals were randomly assigned to one of two groups. Groups C/S and P/S included sham-operated (S) animals that received either continuous reinforcement (C) or 50% partial reinforcement (P) training. Groups C/L and P/L included lesioned (L) animals receiving the same training schedule. Acquisition lasted 32 daily trials and the intertrial interval was approximately 24 hr. All the trials in the C groups ended in reinforcement, with the delivery of 6 pellets of Tetra-pond fish food (about 0.15 g). For the groups receiving 50% partial reinforcement, 16 trials ended in reinforcement, while the rest ended in nonreinforcement. The sequence of reinforced (R) and nonreinforced (N) trials was the following: RNRRN RNRNN RNNRN RNRRN RNNRR NNRNRN RN. Extinction began the day after the last acquisition trial and lasted another 24 daily trials, all nonreinforced.

Animals were transported from their home tank into the start box of the runway. After 20 s, the start door was raised and the trial started. The trial ended when the fish interrupted the photocell located in the goal box. If the trial was reinforced, the food was administered immediately after the photocell was activated and the animal was given 20 s to consume the food. In a non-reinforced trial, animals were left in the goal box during 20 s after the photocell was activated. Animals were returned to their home tanks immediately at the end of each trial. Post-trial supplementary feeding (6 pellets, about 0.15 g) was provided for those animals exposed to an N trial, either during partial reinforcement training or during extinction, 30 min after the end of the trial. The order in which animals received training in any given day was determined as follows: squads of 4 goldfish, one from each group, were run always in the same order, but the order of training within a particular squad was changed randomly across days.

##### *Results*

Three lesioned animals died in the course of the experiment. In addition, one fish in Group P/S failed to complete 5 of the last 8 acquisition trials and exhibit no clear evidence of acquisition. Given the emphasis on extinction performance, this animal with a poor acquisition record was discarded from the study. Only three other goldfish failed to finish a trial (one animal in Group C/S

failed in two trials, and two animals in Group P/L failed in one trial each, out of a total of 32 acquisition trials). Because these were isolated failures, these animals were kept in the analyses. There were, thus, 4 failures out of a total of 896 acquisition trials (only 0.4% disruption). The final group sizes were the following: Group C/S, n=8; Group C/L, n=7; Group P/S, n=7, and Group P/L, n=6.

Fig. 1 shows the extent of the telencephalic tissue removal in representative brains. The lesion resulted in the complete removal of the telencephalic lobes. The preoptic area and the optic tracts were not injured in any of the animals subjected to surgery. None of the sham operated goldfish exhibited damage to the brain.

Latency scores obtained for each animal, in each trial, were transformed to the log<sub>10</sub> for the purpose of improving normality and allowing for parametric analysis of the results. The results are shown in Fig. 2. Early acquisition performance was poorer in both lesioned groups than in the sham controls, but the lesioned animals eventually reached a similar (P/L) or better (C/L) behavioral level than their respective sham controls. There was a small but consistent decrease in latencies in the two sham-operated groups. However, there was no obvious effect of the schedule of reinforcement. A Schedule (C, P) x Lesion (L, S) x 4-Trial Block analysis of variance on the transformed latencies yielded a significant acquisition effect [F(7, 168) = 5.339, p < 0.001], a significant Lesion x Trial interaction [F(7, 168) = 5.835, p < 0.001], and a significant main effect for lesion [F(1, 24) = 4.845, p < 0.04].

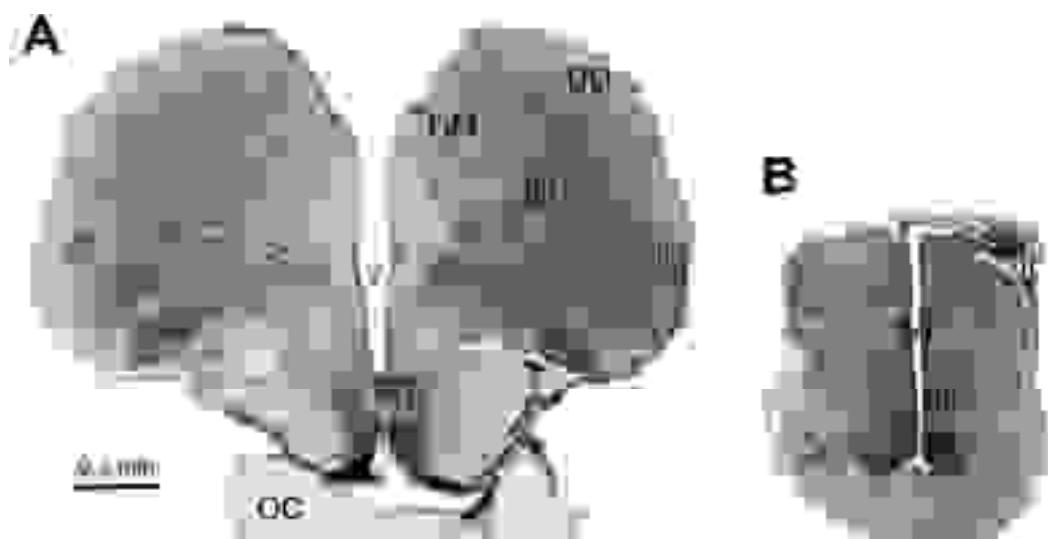
Further pairwise comparisons with the error term and df derived from the main analysis (Howell, 1992) were computed over the last two 4-trial blocks of acquisition in order to determine whether the group differences were significant. Non-significant differences were found in Blocks 7 and 8 between Groups C/S and C/L [Fs(1, 87) ≤ 3.851, all p > 0.05] and between Groups P/S and P/L [Fs(1, 87) < 1, all p > 0.05].

Fig. 2 also shows that both lesioned groups exhibited less persistence in extinction than each of their respective sham con-

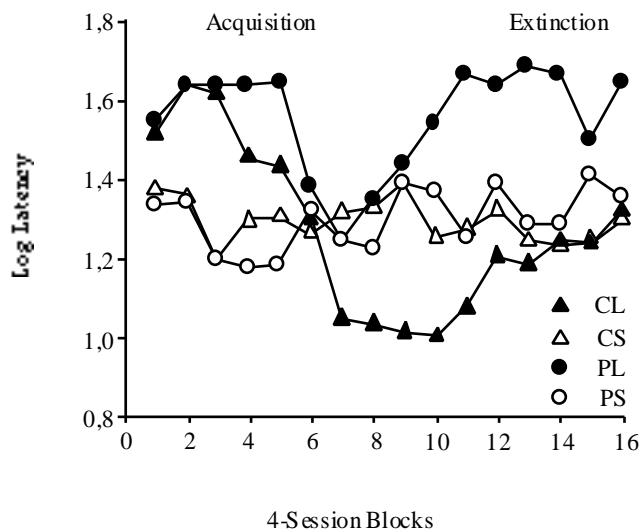
trols. This pattern suggests that the telencephalic lesion affected extinction in the same manner whether the response was acquired via continuous or partial reinforcement training. There was also a tendency for the two groups trained under partial reinforcement to exhibit higher latencies than those of the groups trained under continuous reinforcement. A Schedule (C, P) x Lesion (L, S) x 4-Trial Block analysis of variance on the transformed latencies confirmed both observations. There was a significant lesion by block interaction [F(7, 168) = 2.292, p < 0.03], and a significant main effect of schedule [F(1, 24) = 4.349, p < 0.05].

Although the asymptotic acquisition performance of these groups was statistically similar, it is obvious that the goldfish in Group C/L started extinction at lower latency levels than the other groups. In order to assess extinction rate independently of the starting point, the extinction latencies were re-calculated by using a relative measure. The log<sub>10</sub> latency score of each animal, in each extinction trial, was transformed according to the formula: (trial / asymptote) x 100, where asymptote was the mean log<sub>10</sub> score of that animal during the last 8 acquisition trials. Transformed scores were averaged in blocks of 4 trials for each animal. Thus, scores above 100 represent an increase in latency during extinction, relative to asymptotic acquisition performance.

The results are presented in Fig. 3. There was substantially more change in behavior during extinction, relative to the last 8 acquisition trials, in Groups C/L and P/L than in their relative sham controls, C/S and P/S. There was also a greater average change in the partial reinforcement groups than in the groups that had received continuous reinforcement training. A Schedule (C, P) x Lesion (L, S) x 4-Trial Block analysis of variance on the transformed scores yielded a significant lesion by block interaction [F(7, 168) = 2.307, p < 0.03], confirming that the extinction performance of the lesioned groups extinguished significantly faster than that of the sham controls. There was also a borderline lesion effect [F(1, 24) = 4.072, p = 0.0549]. All other effects were non-significant, including the schedule effects.

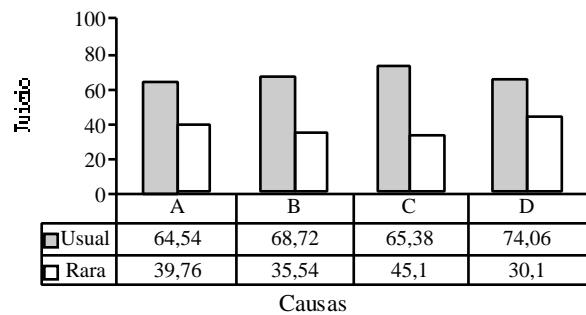


**Figure 1.** Transverse postcommissural section of a normal brain (A) and a telencephalon-ablated brain (B). DC, central area of the dorsal telencephalon; DD, dorsal area of the dorsal telencephalon; DL, lateral area of the dorsal telencephalon; DM, medial area of the dorsal telencephalon; OC, optic chiasm; PO, preoptic nucleus; PP, periventricular preoptic nucleus; tc, tela choroidea; V, ventricle.



**Figure 2.** Performance of groups of goldfish trained under either continuous (C) or partial (P) reinforcement during acquisition, and subject to telencephalic lesion (L) or sham operation (S) before the start of training.

## Experimento 2



**Figure 3.** Extinction performance of groups trained under continuous (C) or partial (P) reinforcement during acquisition, and subject to a telencephalic lesion (L) or a sham operation (S) before training. Latencies (s) from each trial were transformed to their  $\log_{10}$  value before computing the percentage of change. The  $\log_{10}$  value of each extinction trial was divided by the average  $\log_{10}$  value of the last 8 sessions of acquisition and the ratio multiplied by 100. Change above 100 implies longer latencies in extinction than during asymptotic acquisition.

## Discussion

Widely-spaced experiments on instrumental learning with goldfish suggest that behavior is strengthened by reinforcement and weakened by nonreinforcement (Lowes and Bitterman, 1967). Unlike it is the case with mammals, goldfish data, as well as the results of analogous experiments with toads and turtles (Pert and Bitterman, 1970; Muzio et al., 1993; Papini and Ishida, 1994), do not require the assumption that the surprising omission or reduction in reward quantity or quality induces an emotional reaction of frustration (Gray, 1987; Amsel, 1992; Papini and Dudley, 1997). In agreement with previous experiments, there was no evidence of greater resistance to extinction after PR acquisition, rather than CR acquisition, in the sham groups. This was the case whether extinction was assessed in terms of absolute or transformed latencies. The absence of a marked performance change during extinction in the sham groups may appear unusual. However, it is not uncommon for goldfish trained under widely-spaced conditions to exhibit substantial persistence of instrumental behavior during extinction (Boitano and Foskett, 1968; Schutz and Bitterman, 1969).

Lesion experiments can contribute to determine whether behavioral differences across species reflect differences in the underlying processes of instrumental learning. If CR and PR training lead to the same underlying type of learning, as it is hypothesized to be the case in the goldfish, then ablation of a particular area known to be involved in learning should lead to the same extinction effect in both training conditions. The present results demonstrate that the complete ablation of the telencephalon, a structure known to be involved in learning in teleost fish (Overmier and Hollis, 1990), leads to reduced resistance to extinction independently of whether the response was acquired under CR or under PR training.

## Conclusions

- (1) Partial reinforcement training does not increase resistance to extinction in the goldfish.
- (2) A bilateral lesion of the telencephalon does not affect acquisition performance, but it reduces resistance to extinction similarly in animals trained under partial or continuous reinforcement.

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