Optimizing Active Avoidance Conditioning for high-throughput behavioral and cognitive screening in fish

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Introduction

Learning abilities have been widely documented in fishes from experimental studies. For example, a learned foraging behavior in tilapia has been characterized in food-restricted situation using methods such as “time-place learning” [1]. In more natural laboratory conditions, many behavioral or “cognitive ecology” experiments have argued strongly the social facilitation of learning devoted to locate food, avoid predator or gathering information about conspecifics (eavesdropping) [2]. Moreover, different laboratory learning paradigms typically applied in rodents have been successfully validated in fish physiology. Recently, for the first time in fish, a conditioned endocrine response (cortisol) to a stressor was demonstrated in Nile tilapia, using the Pavlovian conditioning [3].

Two-way active avoidance conditioning (“TWAAC”) is another laboratory classical technique chiefly developed for experimental psychopharmacology in rodents. The animal has to cross (at each trial) from one side to the other of a shuttle-box to avoid or escape a mild electrical shock. Thanks to the more suitable ecologically nature of the behavioral response asked to the animal, the active avoidance procedure is tested in fish for a long time [4]. Initially used to compare vertebrate learning performance, TWAAC has become more recently, a deep-rooted behavioral paradigm in goldfish. Well-known model in neuroscience research, the goldfish has permitted to elucidate some questions concerning the neurobiology of learning and memory in the context of simplified vertebrate models approach [5].

However, despite of numerous studies in teleost fishes, conditioning procedures are very few standardized. For example, TWAAC in goldfish is often time-consuming procedure (20 training days/fish) or the trial number per session can differed largely (20-40 trials) [6]. In the context of high-throughput screening for behavioral phenotyping, the optimization and the shortening of conditioning procedures in fish models are in urgent need. In this aim, we present here the major results of three TWAAC experiments in fish that manipulated different parameters: {I} Duration of the InterTrial Interval (ITI) and {II} Nature of the Unconditioned Stimulus (US) in Nile tilapia; {III} Nature of the Conditioned Stimulus (CS) in the classical “goldfish model”.

Materials and Methods

In the two first experiments, we tested a total of 2x20 Nile tilapia (*Oreochromis niloticus*) of 75-80 mm in length, randomly divided into four groups (n=10). For the last experiment, we used a total of 16 goldfish (*Carassius auratus*) of 55-70 mm in length and randomly divided into two groups (n=8).

The 56 fishes were trained and tested individually in four identical fish shuttle box active avoidance systems connected simultaneously to a Smart Control (MED associates, USA). The fish shuttle box consisted of a water-filled tank (41.0 x 20.5 x 10.5 cm) separated by a white hard plastic barrier (20.5 x 10.5 cm) into two equal compartments. There were a white light stimulus and a buzzer (CS) at each end of the tank and four stainless steel electrode plates (18.0 x 19.5 cm) at the top and the bottom of each tank. Two table top shocker modules cabinet delivered customized constant electrical voltage shocks (US). A rectangular opening (6.0 x 3.0 cm) in the barrier permitted fish to swim freely from one side of the tank to the other to escape or avoid the US. The crossing movement of the fish was monitored by four infrared light beams and their corresponding detectors located on the long sides of the tank near the rectangular opening door of the barrier. Additionally, four CCD video cameras were installed in front of each experimental tank to record the training sessions.

Each session was planned with about 20 trials of paired CS/US and the rate (%) of avoidance responses (escape during the CS presentation before the US administration) was an indicator of learning. Two-way Repeated Measures ANOVA was realized for each experiment and multiple comparisons calculated when $p \leq 0.05$.

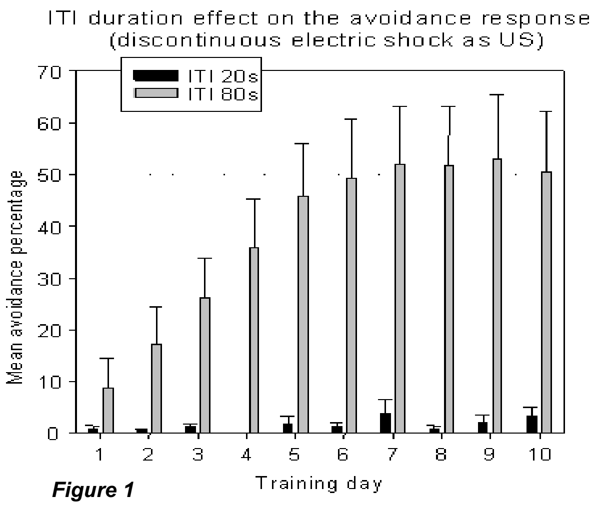

Figure 1

Results and Discussion

{I} Effects of duration of ITI on learning in Nile tilapia.

After the light (CS) was on for 15 seconds (s), a <u>discontinuous</u> mild electric shock (US=10V, pulsed 400 ms on and 600 ms off) was administrated, along with the light, for 15 s. In one group of fishes (n=10), the ITI was 80-s and in the second group (n=10), the ITI was 20-s. The ANOVA indicated a significant session difference [$F(9,171)=7.61$, $p<0.01$] and a strong effect of ITI on learning performances [$F(1,18)=17.54$, $p<0.01$]. The 20-s ITI group did not learn and had very low performances (see Figure 1): the post-ethographic video-analyze has shown more “like-rodent freezing behavior” in these fishes.

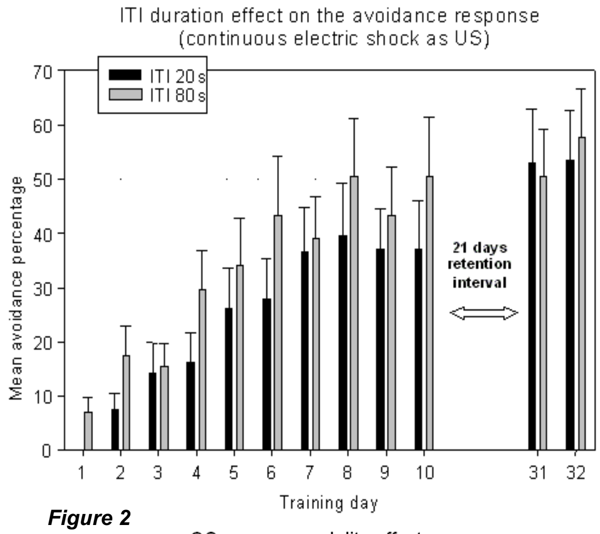

Figure 2

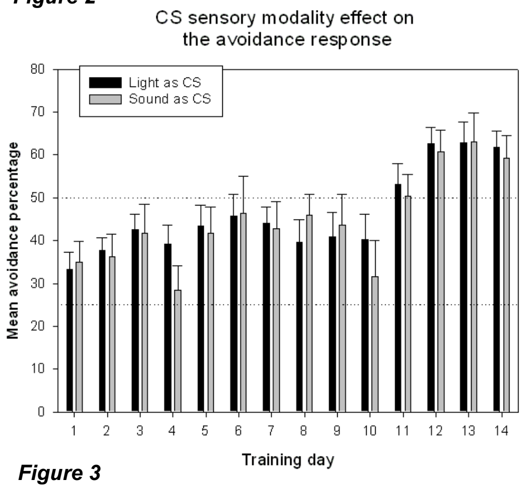

Figure 3

{II}Effects of the Nature of the US on two different ITI: impact on learning and retention in Nile tilapia.

The active avoidance paradigm was the same than in the first experiment, excepted for the US. Here, a continuous mild electric shock (10V, continuous) was administrated to the fish. The ANOVA indicated a significant session difference [$F(10,190)=14.72$, $p<0.01$] and no effect of ITI on learning performances [$F(1,18)=0.95$, $p=0.34$]. After the learning time (10 days), a test of retention was performed after 21 days. This test has demonstrated a long memorization of the task, but also a consolidation with an increase of the rate of avoidance (see Figure 2).

{III} Effects of the nature of the CS in goldfish.

Paradigm: after the CS was on for 20 s, a mild electric shock (US=5V, continuous) was administrated, along with the CS-light (group 1, n=8) or the CS-sound (group 2, n=8), for 20 s. In the two groups, the ITI was 80-s. The ANOVA indicated no difference between the groups trained with a light or a sound as CS sensory modality [$F(1,26)=0.286$, $p=0.60$]. This result would be very useful to design in the future more complex TWAAC procedures such as reversal learning. Furthermore, the pattern of learning was the same in the two groups: light or sound with 80-s ITI conducted to a slow increase of performance (see Figure 3) in goldfish.

Conclusions

This study illustrates the impact of parameters of conditioning procedures on the dynamics of learning. As a result we have found optimal combinations for high-throughput screening in main fish models: a short ITI (20 s) should be coupled with a continuous electric shock adjusted to the species (10 V in Nile tilapia, 5 V in goldfish).

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