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The Effects of Uncontrollable Shocks on the Aggressive Behavior of the Siamese Fighting Fish (*Betta splendens*)

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The aim of this study was to verify the effects of uncontrollable shocks on the aggressive behavior of the *Betta splendens*. Eighteen domesticated *Betta splendens* were divided into 3 groups: Control (CTRL), Controllable Shocks (CSH), and Uncontrollable Shocks (USH). The procedure was performed in 3 phases: (a) register of the aggressive behavior (baseline); (b) treatment with shocks; and (c) register of aggressive behavior (test). The aggressive behaviors registered were display and attack. These behaviors were measured regarding the latency of the first response, response frequency, and duration. The intragroup analysis performed through variances analysis (1-way ANOVA) between the baseline and test session showed significant difference only regarding the latency of the first display response in the test, $H_{13} = 4.078$, $p = .041$, in the USH group, however this response decreased in all the groups, thus this reduction cannot be attributable to the treatment with uncontrollable shocks. Nonetheless, it can be said that, although the other measures didn’t present statistical significance, there was a tendency for the aggressive behavior in the Group USH to diminish, because the display duration, frequency of display, and attack decreased, and the latency of the first attack increased, all of these results were expected to the USH group. Thus, we concluded that taking into account the experimental conditions of this study, the uncontrollable shocks do not attenuate the aggressive behavior of *Betta splendens* in the same degree they do to rodents, possibly as a result of methodological differences between the studies.

**Keywords:** aggressive behavior, *Betta splendens*, uncontrollable shocks

Agonistic behavior is considered any behavior of individuals from the same species that involves fight and dispute for environmental resources (King, 1973; Price, 1969); it is a behavioral system which has the function to adapt to conflicts between conspecifics (Scott, 1966). According to King (1973), and Price (1969), agonistic behavior involves threatening the opponent, attack, retaliation, and escape responses, and the like. It is a dynamic behavior pattern in which the individuals express both offensive and defensive responses during the interaction.

Among the stimuli that control the agonistic behavior are some internal and external determinants; examples of internal determinants are hormone levels, genotype, and neurological factors. However, the external determinants vary among the species, nonetheless they are related to environmental changes and behaviors of other conspecifics. King (1973) states that changes in the external environment modify the internal state of the organism, making it predisposed to engage in different levels of agonistic interactions. Therefore, the environmental changes may not only lead to agonistic behavior but also be produced by it (King, 1973).

Even though agonistic behaviors are commonly observed in several species, according to researchers of the area (King, 1973; Price, 1969; Scott, 1969).
The agonistic confrontation between males of *Betta splendens* aggressive behavior has been investigated, at least since the 1960s decade, through learned helplessness which is defined as a deficit in learning new responses due to a previous contact with uncontrollable shocks. However, some researchers have investigated the role of shock uncontrollability on unlearned behaviors, such as aggression elicited by shock (Maier et al., 1972; Payne et al., 1970) and territorial aggression (Corum & Thurmond, 1977).

The experiment of Payne et al. (1970) compared groups of rats that were pretreated with shocks previously to a test session when the subjects could attack each other with paws and teeth. The shocks were delivered in two different intensities (2 and 5 ma) and intervals (1.5 and 19.5 s) for 3 minutes, daily, for 5 days; there were also groups that didn’t receive shock (control group). The results show that those animals previously exposed to uncontrollable electric shock showed decreased aggressiveness on the test session.

Nonetheless, Maier et al. (1972) investigated the effect of uncontrollable electric shock comparing rats that received controllable shocks, uncontrollable shocks, and others that didn’t receive shock (Control Group); the one that received uncontrollable shock was yoked to the one that received controllable shocks, hence receiving the same amount of shocks. The results showed that from the 50 shocks received on the test session, when the subjects could attack each other, the animals from the uncontrollable group responded aggressively to 28 shocks in average, whereas the ones that received controllable responded to 36 shocks, and the group that didn’t receive previous shocks.
responded to 38 shocks in average. Therefore, the authors demonstrated that uncontrollable shocks produce much more deficit on aggressive behavior than the no shock or controllable shock conditions.

Corum and Thurmond (1977), on their turn, performed an experiment using mice in a territorial aggression test, in which they observed the effects of uncontrollable shocks on attack to intruders. The animals were separated into three groups: controllable shock, uncontrollable shock, and no shock. The results showed that the numbers of attackers was significantly smaller in the uncontrollable shock group when compared with the control group ($p < 0.05$). The results of this study, therefore, highlight the harmful effects of previous exposure to uncontrollable shocks on the later aggressive behavior.

Acknowledging such data with rodents and the lack of studies in the literature that propose to perform a systematic investigation of the effects of uncontrollable shocks on the agonistic behavior of *Betta splendens*, this study was proposed to verify whether the aggressive behavior will suffer deficits after receiving uncontrollable shocks, similarly to what happened to mice and rats on previously described studies, and as it happens in learning, consequently broadening the knowledge on the effects of lack of control on unlearned behaviors, more precisely, the agonistic behavior of *Betta splendens*.

**Method**

**Subjects**

Eighteen *Betta splendens*, male, domesticated, blue-colored fish, were acquired at a local pet shop (Belém, Pará, Brazil). The subjects were kept individually in aquarium for at least one week before the procedures, in constant conditions: temperature of $26 \pm 2^\circ C$, light cycle 14/10 h (starting at 6 a.m.). The animals were fed with flake food (Minibetta, Tetra, Germany) once a day. The animals were divided into three groups, depending on the type of shock treatment: Controllable Shock Group (CSH); Uncontrollable Shock Group (USH); and Control Group (CTRL).

**Equipment and Materials**

**Register of aggressive behavior.** Glass aquarium measuring 7.5 cm long, 11 cm wide, and 14 cm high; a plain mirror with 9.8 cm wide and 14.8 cm high, an opaque barrier that covered the mirror before the recording, and a water column of 9 cm.

**Shock treatment.** Aquatic active-avoidance box (Insight), made of transparent acrylic, measuring 42 cm long, 15 cm wide, and 12 cm high. Inside this apparatus there was a gray colored half octagon shaped wall. There were also photoelectric sensors installed in the bottom and on the cover of the box, in parallel, linked to the front wall of the apparatus. On the smaller walls there were metal plates, in parallel, connected to an electric shock generator, from which the electric shocks were delivered. This generator had the controls for the shock voltage, the percentage of luminous and sound intensities, and a key to select between alternated or constant current. The box, the generator, and a laptop (SONY VAIO VPCCW13FB) containing the software that programmed the sessions were all connected.

For the data collection the active avoidance box was reset in a way that the metal plates through which the shocks were delivered were placed side by side instead of in parallel, separated by one of the walls from the half octagon; and the area where the animal was placed was reduced to 12 cm long, 3.5 cm wide, and 12 cm high. For the USH group a barrier was placed in the center of this area to impede the escape response of the animal. The data collection from all the groups was performed in the changed apparatus and the water column was of 5.5 cm.

**Procedure**

The procedure was performed in three phases: Phase 1, Register of aggressive behavior in baseline; Phase 2, shock treatment; and Phase 3, Aggressive behavior test. The CSH and USH groups went through the three phases, but the CTRL group only went through Phases 1 and 3, not being exposed to shock.

In Phase 1 the animal was placed in the apparatus for the register of aggressive behavior, after 10 min habituation the opaque barrier that covered the mirror was removed, then the aggressive behavior of the animal was recorded for 10 min. From the 10 minutes recorded, only the 5 minutes in which the animal was facing the mirror were transcribed.
The registered categories of aggressive behavior were twofold: (a) the display (D) and (b) the attack (A); these categories were based on the studies of Bronstein (1984, 1985), de Matos Mansur, Cavalcante, Santos, and Gouveia Jr. (2012), Fantino, Weigele, and Lancy (1972), and Simpson (1968). The category biting described in some of these studies was replaced by attack in this study, for it was considered more appropriate because of the response topography. The display is marked by the horizontal or vertical movements with aperture of dorsal fin, caudal fin, gill cover erection; the attack corresponds to rapid movements toward the mirror with or without mouth aperture.

The display was transcribed by the measures of three analysis unit: latency of the first response, frequency of response and total duration. For the attack category only the frequency and latency of the first response were measured. The frequencies were registered with the aid of the EthoLog (Ottoni, 1996) software, however the time was manually measured due to a technical problem with the software that didn’t provide those data on the transcription report.

Phase 2 was accomplished approximately 24 h after Phase 1. For the CTRL group this phase consisted only in placing the animals in the active avoidance box without receiving shocks; they stayed in the box for about 25 min. For the CSH group the animals were placed in the box and after 10 min habituation the shocks started being delivered, composed by constant current, and 0.6V voltage, approximately; if the animal escaped the shock, crossing to the other side of the box, it was immediately turned off, if it didn’t escape it would last 30 seconds maximum. The total of three shocks were delivered in variable intervals of 18 s, 40 s, and 80 s. The shock delivery and the escape response latencies register were performed manually because of technical problems of the equipment.

In this phase, for the CSH Group the criteria for closing the session was three consecutive escapes, for the domesticated Betta splendens (strain used here) has a tendency to react to aversive stimuli with immobility response (Verbeek, Iwamoto, & Murakami, 2008). Previously in our laboratory six pilot studies were performed and it was observed that the animals escaped the shock only three times, in average, remaining still when received the other shocks; in the pilot-studies each Session 15 to 30 shocks were delivered. Therefore, the criterion was adopted to guarantee the existence of a control group with controllable shocks to be used as comparison. It is noteworthy that the no animal stayed still during the delivery of each of the three shocks.

Phase 2 for the USH Group consisted in pairing the animals from this group to the ones in CSH Group, in a way that each animal of the first group also received three electric shocks that had duration corresponding to the animals of the second group. However, the latencies shorter than 1 s were rounded off to this value, as the software doesn’t enable programming the shock duration in milliseconds and the values under 1 s were not trustingly recorded manually. The CSH Group values and its pairs from USH are represented in Table 1.

Phase 3, the aggressive behavior test, took place approximately 24 h after the shock treatment, the procedure was the same from the Phase 1 when the aggressive behavior was registered in baseline. The transcription was also the same.

**Statistical Analysis**

The statistical analysis was made intragroup and between groups. For the intragroup analysis

### Table 1

**Values of Electric Shock Duration From CSH and USH Groups in the Tree Trials**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Trials</th>
<th>Shock duration (s)</th>
<th>Subjects</th>
<th>Trials</th>
<th>Shock duration (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSH1</td>
<td>1</td>
<td>0.43</td>
<td>USH1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>6.75</td>
<td></td>
<td>2</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.88</td>
<td></td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>CSH2</td>
<td>1</td>
<td>0.46</td>
<td>USH2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>0.50</td>
<td></td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.71</td>
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<td>3</td>
<td>1</td>
<td></td>
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<tr>
<td>CSH3</td>
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<td>USH3</td>
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<td>1</td>
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<tr>
<td>2</td>
<td>0.91</td>
<td></td>
<td>2</td>
<td>1</td>
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<tr>
<td>3</td>
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<td></td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
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<td>0.68</td>
<td>USH4</td>
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<td>1</td>
</tr>
<tr>
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<td>0.69</td>
<td></td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
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<td>0.44</td>
<td></td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
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<td>1.88</td>
<td>USH5</td>
<td>1</td>
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</tr>
<tr>
<td>3</td>
<td>5.40</td>
<td></td>
<td>3</td>
<td>6</td>
<td></td>
</tr>
<tr>
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<td>USH6</td>
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<td>1</td>
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<td>2</td>
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<td>3</td>
<td></td>
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<tr>
<td>3</td>
<td>2.25</td>
<td></td>
<td>3</td>
<td>3</td>
<td></td>
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</table>
the data were analyzed comparing the sessions (baseline and test) through one-way ANOVA (factor session) when the data reached the normality criteria, and Kruskal–Wallis nonparametric test when such criteria was not reached. The analysis between groups was made comparing the two sessions through Kruskal–Wallis test. The value for statistical significance was $p < .05$.

### Results

The intragroup analysis showed that from the measured units (latency of the first response, duration and frequency), the only one that presented statistically significance difference was the latency of the first display response in the USH Group, $H_{(1)} = 4.078, p = .041$, which decreased from an average of 24.5 $\pm$ 35.84 in baseline to 2.16 $\pm$ 2.56 in the test. Figure 1 shows the data of average latency of the first display response in reference to the baseline and test sessions of CTRL, CSH, and USH groups.

The total display duration did not present significant statistical difference between the sessions in any of the three groups, but it was reduced in the test sessions in all the groups (see Figure 2).

The display frequency also did not present statistical significant difference between the session in any of the groups. Figure 3 shows the data of display frequency of the groups across the sessions.

The attack responses, either its frequency or latency of the first response, didn’t present significant statistical difference between baseline and test sessions in any of the groups. Figure 4 shows the frequency of these responses, and Figure 5 shows the latency of the first attack response.

The Kruskal–Wallis test in the analysis between groups did not show any statistically significant difference in baseline and tests sessions for any of the responses in any of the groups, the $p$ values were higher than 0.05.

### Discussion

Based on these results, it cannot be said that the electric shock uncontrollability attenuated the aggressive behavior of the Siamese fighting fish in the same degree as it occurs to rats and mice (Corum & Thurmond, 1977; Maier et al., 1972; Payne et al., 1970), demonstrated by the lack of intragroup significant statistical difference (in baseline and tests), and between groups...
(Control, Controllable Shock, and Uncontrollable Shock)—with the exception of one single response in the intragroup analysis—for none of the aggressive responses measured.

It was observed that the only category of aggressive behavior from Betta splendens that showed significant statistical difference was the latency of the first display response in the USH Group, in which the average decreased from $24.5 \pm 35.84$ in baseline to $2.16 \pm 2.5$ in the test. Nonetheless, as there was a decrease in all three groups in this measure, the statistical significance cannot be attributable to the treatment with uncontrollable shock. Such decrease can be explained by the Betta splendens’ aggressive behavior dynamic; on a second exposure, either to their own image on a mirror or to a conspecific, it is observed a decrease in the latency to begin approaching the opponent (Matos & McGregor, 2002).

Nonetheless, in spite of the absence of statistical significance, there was a tendency for the aggressive behavior in the Group USH to diminish, given that by looking at the figures it can be seen a decrease in the display duration, frequency of display and attack, and the latency for the first attack increased, all of these results were expected to the USH group, and happened accordingly, even though none presented statistical significance.

One of the possible explanation is the methodological differences between this study and those performed with rodents, for instance a higher numbers of shocks delivered to the animals, the use of yoked groups to guarantee that the animals receive the same amount of shocks, and the selection of a response that is not incompatible with the escape response planned.

Others possible hypotheses may be the intragroup variation is too high, which indicates that there are several individual differences (the high values of standard deviation shows that); hence, it is necessary a higher $n$ to balance the sample, thus decreasing the intragroup variation. With such decrease the probability of having significant differences intragroup and between groups would increase, and then different results would be attributable to different treatments with electric shock.

On the other hand, the second hypothesis is that Betta splendens’ aggressive behavior is so stable that even a condition considered to be much stressed as to receive uncontrollable electric shock is incapable of attenuating it in a way that the animal drastically decreases or stop reacting aggressively facing an opponent inside its territory.

To support this hypothesis there are some studies that investigated the effects of some variables on Betta splendens’ agonistic behavior and there were no differences found regarding the control animals. de Matos Mansur et al. (2012) investigated the effects of mercury chloride on the aggressive behavior of the Siamese fighting fish and observed that the typical aggressive responses as gill cover erection, attacks, and vertical display were not changed after the drug treatment. Lynn et al. (2007) exposed Betta splendens to fluoxetine treatment at $3 \mu g/ml$ for 3 h and didn’t observe significant reduction when compared with the control group, only three responses were measured from all the behaviors comprised in the aggres-
sive behavior, latency of the first response, gill cover erection and display, the only one changed significantly was the display.

Dore et al. (1978) verified two different water conditions: (a) An aquarium with water where a conspecific had exhibited aggressive behavior; and (b) An aquarium where a conspecific had been injured in an interaction) on the aggressive behavior of Betta splendens during a mirror test and observed that no aggressive response were decreased when compared to animals tested in unaltered water. Meliska and Meliska (1976) investigated whether continued exposure to a mirror or to a conspecific would change the Siamese fish during a dominance test, and it was observed that the exposure to 14 h for 12 consecutive times to the mirror, and to 14 h for 26 consecutive times to conspecifics didn’t decrease the behavior during the test as an effect of habituation.

Therefore, considering the data of the previously described experiments, it can be observed that the Betta splendens agonistic behavior presents a considerable stability, which makes it harder to be changed. This fact can be seen as an advantage for the species; as it is a very stable behavioral pattern, it can be inferred that only major aversive events are capable of attenuating it, which makes sense for it is a pattern that also evolved as reproductive strategy (Bronstein, 1984). Thus, it would not be adaptive to the animal, considering the species, that such behavior would be too sensitive to general stressors. Considering the current experiment with electric shock maybe higher voltage and different schedules of shock presentation are necessary to attenuate this behavior, thus bringing more light on the effects of uncontrollable shocks on this behavior of this particular species.

Hence, acknowledging such results, it can be said that uncontrollable electric shocks tend to attenuate the aggressive behavior of Betta splendens; however, considering the experimental settings used, the shocks do not attenuate this behavior the same way they do to rodents.

We emphasize the need for more studies about the effects of electric shocks on the aggressiveness of Betta splendens making use of methods similar to those used with rodents, such as yoked groups, and to use a more appropriate response for this species to avoid incompatibilities with the planned escape response.

References


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