Fish Physiol Biochem (2017) 43:755–759
DOI 10.1007/s10695-016-0329-9

Acute fluoxetine treatment increases aggressiveness in juvenile matrinxã (*Brycon amazonicus)*

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Received: 3 August 2016 / Accepted: 11 December 2016 / Published online: 24 December 2016

Abstract Fluoxetine (FLX) is a selective serotonin (5-HT) reuptake inhibitor known for its effects modifying aggressiveness, personality traits, and anxiety-like behaviors. The aim of the present study was to evaluate the influence of the acute treatment, by immersion, with FLX on aggressive behavior of resident *Brycon amazonicus* fish. Fish pretreated with FLX presented an increase in aggressiveness, evidenced by the increase on the number of bites and chases against the intruder and a decrease in latency for the first attack, when compared to control fish. Together with previous studies, these results show the complexity of the neural modulation of the aggressive behavior in fish by 5-HTergic system.

Keywords Aggressive behavior · Fish · Fluoxetine · Serotonin

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Introduction

Numerous pharmacological, neuroanatomical, and clinical studies have suggested that serotonin (5-hydroxytryptamine or 5-HT) plays a critical role in modulating some dimensions of personality and behavior and may contribute to the symptoms observed in a wide range of related psychiatric disorders. Thus, there is substantial evidence implicating the serotonergic (5-HTergic) system as a mediator of emotional responses in animals and humans (for a review, see Gillette 2006). 5-HT primarily plays an inhibitory role in the expression of aggression and has been shown to influence the dynamics of agonistic interactions (Nelson and Chiavegatto 2001).

The evidence that the 5-HTergic system is an important modulator of mood and emotions and controls many other kinds of behaviors and physiological functions comes from pharmacological manipulation studies. The two most common methods to investigate the role of 5-HT on the modulation of aggression are the employment of selective 5-HT reuptake inhibitors (SSRIs), such as fluoxetine, paroxetine, and sertraline, and dietary supplementation with the amino acid precursor of 5-HT L-tryptophan (TRP). TRP supplemented diets have been linked to decreased aggression in several fish species (Winberg et al. 2001; Hseu et al. 2003; Lepage et al. 2005; Höglund et al. 2005; Wolkers et al. 2012, 2014). Recent studies from our laboratory using South American freshwater fish matrinxã (*Brycon amazonicus,* Spix and Agassiz) as a model organism demonstrated that dietary TRP supplementation for 7 days inhibited aggressiveness against a same-sized intruder, without significant alterations in hypothalamic

5-HT levels (Wolkers et al. 2012, 2014). However, there are contradictory findings concerning the effects of SSRIs in the modulation of aggression. Both acute and chronic fluoxetine administration decrease aggressive behavior in bluehead wrasse (*Thalassoma bifasciatum,* Bloch) males (Perreault et al. 2003). Conversely, acute but not chronic treatment with fluoxetine increases aggression in *Betta splendens* (Clotfelter et al. 2007) and toadfish (*Opsanus beta*; McDonald et al. 2011).

Matrinxã (*B. amazonicus*) is an appropriate model organism to study aggression, as they display extreme aggressive behavior against intruders (Wolkers et al. 2012, 2014). Based on previous studies from our group that demonstrated decreased aggression in fish fed with the 5-HT precursor L-TRP (Wolkers et al. 2012, 2014), we hypothesize that treatment with fluoxetine, an SSRI, could also decrease the aggressive behavior of this species using the same resident-intruder paradigm. Thus, the aim of the present report was to evaluate the effect of acute treatment with fluoxetine on aggression in matrinxã.

Materials and methods

Experimental treatments

To evaluate the influence of the 5-HTergic system on aggressive behavior in *B. amazonicus*, we used the administration of fluoxetine, a selective serotonin reuptake inhibitor. The aggressiveness of the resident fish against the intruder after being treated with either water (Control group) or fluoxetine (FLX) during a 20-min fight was tested.

For FLX treatment, a noninvasive method was used with the addition of the drug into the water of the aquarium in order to avoid stress induced by manipulation. For this purpose, a stock solution was prepared containing 20 μg mL^{-1} of FLX. Twelve hours before the social challenge, 25 mL of this solution were added per liter of aquarium water, resulting in a final nominal concentration of FLX of 0.5 mg L^{-1}.

Experimental protocol

Before the experiments, a total of 28 fish were collected from the tanks, anesthetized (benzocaine, 100 mg L^{-1}, Synth, Diadema, Brazil, www.labsynth.com.br), weighed and measured, and then randomly separated into two groups (residents and intruders). For identification during interactions, animals were also tagged with a small cut on the superior (resident) or inferior (intruder) lobe of the caudal fin. The resident fish were randomly separated in two treatments, control (n = 7) and FLX (n = 7), and they were kept on individual aquaria for 9 days of acclimation. Intruder fish were kept in individual tanks for the same period with the same water as the control group. On day 9, 12 h prior to the test, an FLX solution or vehicle was included in the resident fish aquaria. On day 10, one intruder fish was introduced into the aquarium of each resident to establish a resident/intruder relationship. Residents and intruders were similar in mass and total length (differences of less than 10%). The behavioral interactions were recorded for 20 min immediately after the entrance of the intruder fish. The resident fish were anesthetized, and blood was collected by caudal vein puncture for subsequent biochemical analyses immediately after the end of the experiment. All samplings were performed between 8:00 and 11:00 a.m.

Behavioral and biochemical analysis

The agonistic behavioral patterns of the resident fish were analyzed by observing the occurrence of biting (characterized by a bite on the other fish, on any part of the body) and chasing (characterized by an explosion of locomotor activity in which one fish pursued the other without body contact) (adapted from Wolkers et al. 2012). The latency before the first attack was also analyzed. The serum cortisol levels were evaluated by radioimmunoassay (DPC kit, Diagnostic Products Corporation).

Statistical analysis

The data presented a normal distribution (Kolmogorov–Smirnov test, $P > 0.05$) and homogeneity of variance (Levene's test, $P > 0.05$) and are expressed as mean ± standard error (S.E.). T test was performed to determine the effects of the FLX treatment on the latency to the first attack, number of bites and chases, and on the cortisol levels. Only the data of resident fish were considered.

Results

Fish treated with FLX performed more bites ($t = -3.432$, $P = 0.005$) and chases ($t = -2.578$, $P = 0.024$) than control (Fig. 1). Additionally, FLX-treated animals had a smaller latency to the first attack than control group ($t = 2.504$, $P = 0.028$) (Fig. 2). The serum cortisol was not affected by the treatment ($t = 1.637$, $P = 0.124$) (Fig. 3).

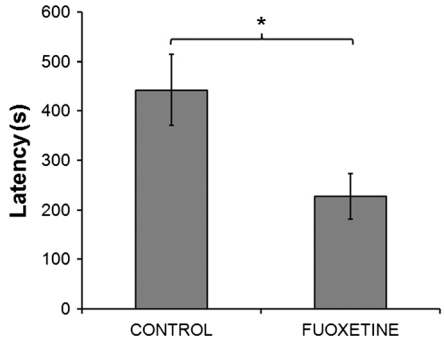

Fig. 2 Latency (seconds) to the first attack of resident *B. amazonicus*. *Control* represents fish treated with water. *FLX* represents fish treated with fluoxetine (0.5 mg L^{-1})

Discussion

The present report demonstrates that 12 h of immersion in FLX solution, an acute treatment, promoted a significant increase in aggressiveness of juvenile matrinxã compared to the control group. This result was contradictory to our hypothesis, which was based on several studies showing an inhibition of aggression after FLX treatment (Perreault et al. 2003; Lynn et al. 2007; Kania et al. 2012; Forsatkar et al. 2013). Furthermore, cortisol levels after 20 min of fighting with a same-sized conspecific intruder were consistent with the literature (Wolkers et al. 2014; Serra et al. 2016), with no significant difference between the treatments.

Fighting is stressful for fish (Øverli et al. 1999, 2004), and even after 12 h of FLX treatment, matrinxãs were able to properly respond to this stressor, reaching cortisol levels that were similar to the control group and consistent with cortisol levels observed in matrinxã (control fish) after a similar fight paradigm demonstrated in previous studies (Wolkers et al. 2014; Serra et al. 2016). In jundiá *Rhamdia quelen* juveniles, a short (17 min) exposure to FLX in the water also altered the behavior (had an anxiolytic effect) without impairing the stress response (Abreu et al. 2016). Our study also demonstrated that cortisol levels were unaffected by the treatment with FLX, with both groups presenting similar hormonal levels to those described in a previous study that used FLX intraperitoneal implants for an acute treatment (McDonald et al. 2011).

The acute effect of SSRIs consists of the inhibition of the 5-HT transporter (SERT) that removes the 5-HT from the synaptic cleft back into the presynaptic neuron, leading to a consequent longer availability of synaptic 5-HT (Beasley et al. 1992). This increase in the 5-HT availability promoted by the FLX treatment has been related to decreased aggressiveness in fish. In coral reef (*T. bifasciatum*) and betta fish (*B. splendens*), chronic and acute FLX treatments were effective in reducing aggressiveness (Perreault et al. 2003; Lynn et al. 2007;

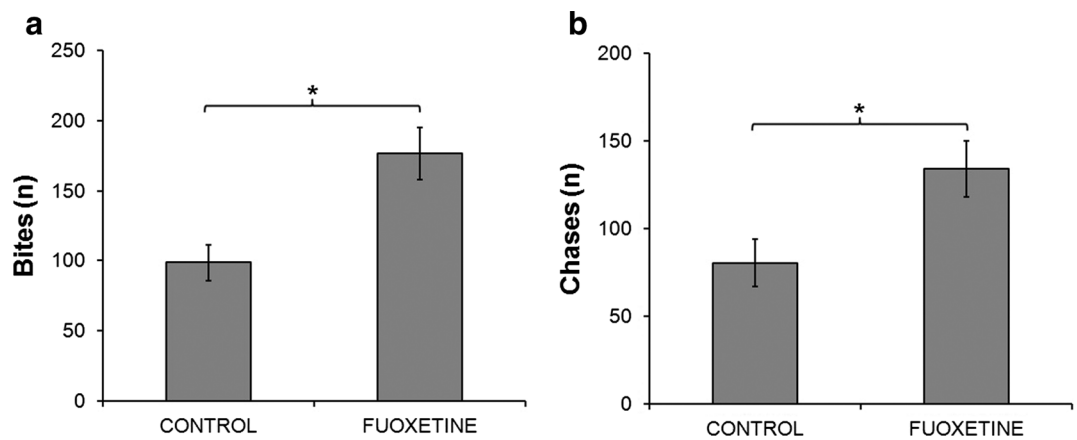

Fig. 1 Aggressive behavior of resident *B. amazonicus*. **a** Number of bites. **b** Number of chases. *Control* represents fish treated with water. *FLX* represents fish treated with fluoxetine (0.5 mg L^{-1})

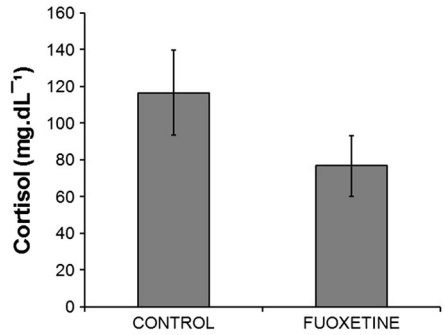

Fig. 3 Serum cortisol (mg.dL^{-1}) of resident *B. amazonicus*. *Control* represents fish treated with water. *FLX* represents fish treated with fluoxetine (0.5 mg L^{-1})

Kania et al. 2012; Forsatkar et al. 2013). However, the decrease of aggression in fish treated with FLX is not universal. Clotfelter et al. (2007) demonstrated that chronic intraperitoneal FLX (14 days) treatment did not decrease the aggression against a mirror image, and promoted a decrease in 5-HT and 5-hydroxyindoleacetic acid (5-HIAA, the 5-HT metabolite) concentrations. Similarly to what was observed in the present study, the acute treatment with intraperitoneal FLX implants enhanced aggressiveness in dominant toadfish (*Opsanus beta*), even with increased circulating levels of 5-HT (McDonald et al. 2011).

Although it is difficult to compare the dosage between the studies, considering the differences in drug administration pathways, the increase in aggressiveness seems to be related with acute FLX treatments, even with increased levels of plasmatic 5-HT, as observed by McDonald et al. (2011), suggesting that the increase in 5-HT levels is not per se an inhibitor of aggression and that other mechanisms may be involved in the modulation of the aggressiveness. Furthermore, the increase in 5-HT plasmatic levels described by McDonald et al. (2012) does not exclude the possibility of a decrease in this neurotransmitter within specific brain regions through negative feedback by 5-HTergic autoreceptors (5-HT$_{1A}$ and 5-HT$_{1B}$), which could influence behavior. However, since the 5-HT levels were not evaluated in the present study, it is not possible to confirm this hypothesis.

Another hypothesis for the increased aggressiveness observed is the influence of FLX on anxiety and boldness. FLX is a drug with anxiolytic effects that have been reported in fish (Lynn et al. 2007; Maximino et al. 2011; Barbosa Júnior et al. 2012; Abreu et al. 2016), and lines selected for low anxiety-related behavior are more aggressive than lines with high anxiety-related behavior or nonselected mice (Neumann et al. 2010). Furthermore, FLX treatment can also improve the boldness in fish (Winberg and Thörnqvist 2016), and this effect can be related to increased aggressiveness, as proactive animals tend to be more bold and aggressive (Benus et al. 1991). Additionally, bold fish are even more likely to become dominant (Dahlbom et al. 2011). However, the methodological approach used here is not adequate to evaluate the effects of FLX on these personality traits and their relationship with aggressiveness.

Conclusion

The present study concludes that modulation of the 5-HTergic system through the acute treatment with FLX increased aggressiveness in matrinxã juveniles. Further studies should be made to investigate which mechanisms drive this increase in aggressive behavior in the species after an acute modulation of the 5-HTergic system with FLX.

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