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Prozac affects stickleback nest quality without altering androgen, spiggin or aggression levels during a 21-day breeding test

Marion Sebire[a,*], Jessica Elphinstone Davis[a], Robert Hatfield[a], Svante Winberg[b], Ioanna Katsiadaki[a]

[a] Cefas Weymouth Laboratory, Barrack Road, The Nothe, Weymouth, Dorset DT4 8UB, United Kingdom
[b] Uppsala University, Department of Neuroscience, Box 593, 751 24 Uppsala, Sweden

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ABSTRACT

Pharmaceuticals are increasingly being used in human and veterinary medicine, and their presence in the aquatic environment may present a threat to non-target aquatic organisms. The selective serotonin reuptake inhibitor fluoxetine (Prozac) has been reported to affect diverse behaviours (feeding, aggression, and reproduction) and also the endocrine system (steroid biosynthesis pathway) in fish. To investigate these claims further, and in particular effects on androgen synthesis, male three-spined sticklebacks (*Gasterosteus aculeatus*) were exposed to fluoxetine at 0, 3.2, 10 and 32 μg/L in a flow-through system for 21 days. Their sex was determined prior to exposure using a non-invasive method to collect DNA for determining the genetic sex, reported here for the first time. This was necessary as the exposure required males of a non-breeding status which had not developed secondary characteristics. Post exposure a number of biochemical (serotonin, steroid and spiggin levels) and apical (aggressive behaviour) endpoints were measured. No effects were detected on morphometric parameters, spiggin or androgen (11-ketotestosterone) levels. However, all fluoxetine-exposed male fish had higher cortisol levels in comparison to the control fish, although this effect only persisted throughout the whole exposure duration at the highest concentration (32 μg/L). In addition, the ratio of 5-HIAA/5-HT (serotonin metabolite/serotonin) was significantly lower in the brains of males exposed to fluoxetine at all concentrations tested. Although we found no differences in the number of nests built by the males, the quality of the nests produced by the fluoxetine-exposed males was generally inferior consisting only of a basic, rudimentary structure. Males exposed to 32 μg/L of fluoxetine displayed a delayed response to a simulated threat (rival male via own mirror image) and were less aggressive (number of bites and attacks) toward their mirror image, but these differences were not statistically significant. In summary, fluoxetine exposure resulted in reduced serotonergic activity in the male three-spined stickleback brain suggesting that the mechanism of action between humans and fish is at least partially conserved. Furthermore, this study provided additional evidence of cross-talk between the serotonergic and stress axes as demonstrated by the perturbations in cortisol levels. This potentially complex interaction at brain level may be responsible for the effects observed on nest quality, an endpoint with serious ecological consequences for this species. Finally, despite our hypothesis (an effect on steroid biosynthesis, based on limited literature evidence), we observed no effects of fluoxetine exposure (at the concentrations and duration employed) on male stickleback androgen levels.

1. Introduction

Antidepressants are now one of the most commonly prescribed medications in the United States and the United Kingdom (National Center for Health Statistics, 2015; Scholes et al., 2014). Their main use is to alleviate mood disorders, such as major depression, dysthymia and social anxiety disorders. Based on their mechanism of action, antidepressants were classified into five major classes: monoamine oxidase inhibitors (MAOIs), tricyclic antidepressants (TCAs), tetracyclic antidepressants (TeCAs), serotonin-norepinephrine reuptake inhibitors (SNRIs) and selective serotonin reuptake inhibitors (SSRIs). The last group is by far the most commonly prescribed and their effectiveness and adverse effects are the subject of many studies and competing claims.

* Corresponding author.
E-mail address: marion.sebire@cefas.co.uk (M. Sebire).

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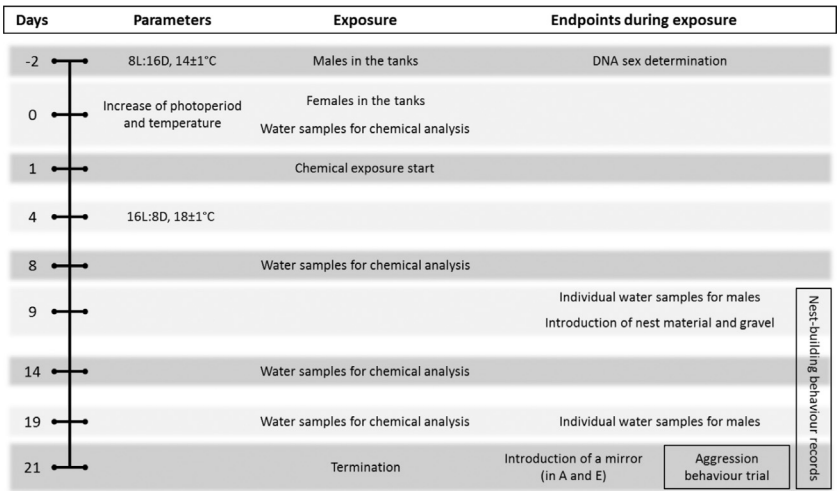

Fig. 1. Schematic summary of the experimental protocol.

According to the statistical analysis of NHS prescriptions for 2013 in England, the number of prescriptions for a single antidepressant drug alone (Citalopram Hydrobromide, a SSRI) was 13 million (among the 20 most prescribed drugs). In total, >53 million prescriptions were made for antidepressant drugs.

Pharmaceuticals are generally designed to resist rapid metabolism (leading to low environmental degradation). Antidepressants are no exception to this rule and have very low degradation rates (Redshaw et al., 2008). They have been detected in water and sediment (Schultz and Furlong, 2008; Styrishave et al., 2011; Vasskog et al., 2008) and fish tissues (Brooks et al., 2005; Chu and Metcalfe, 2007). In addition, persistence and bioaccumulation of fluoxetine (a SSRI) and norfluoxetine (its active metabolite) in fish were greater than their predicted half-life estimates, derived from using mammalian species (Paterson and Metcalfe, 2008).

SSRIs block presynaptic serotonin reuptake transporters (SERT), have a low affinity to noradrenaline uptake sites and very low affinity for neurotransmitter receptors (Ciraulo et al., 2011; Frazer, 1997). Both serotonin and its receptors have been identified in a number of fish species (Caamaño-Tubío et al., 2007; De Lucchini et al., 2001; Winder et al., 2009; Yamaguchi and Brenner, 1997) and most importantly serotonin appears to be involved in a number of critical physiological processes such as behaviour (aggression, social status), immunomodulation, and reproduction (Alanärä et al., 1998; Khan and Deschaux, 1997; Øverli et al., 2005, 1998; Winberg et al., 1997, 1992a, 1991). The *in vivo* studies evaluating the effects of exposure to antidepressants on fish physiology and behaviour are limited. SSRIs have been studied in more detail than the other antidepressants and there are reports of alterations of territorial (Perreault et al., 2003; Semsar et al., 2004) and feeding (Gaworecki and Klaine, 2008; Stanley et al., 2007) behaviours, although other studies did not reach the same conclusions (Foran et al., 2004; Schultz et al., 2011). Fluoxetine is a very common SSRI antidepressant drug known as Prozac, which was first introduced in the United States in 1988 and is now widely used in many countries (Ciraulo et al., 2011). A recent report outlined the presence of fluoxetine in more than 50% of the 162 wastewater treatment works sampled at concentrations exceeding the relevant EQS (average of 23 ng/L; Gardner et al., 2012).

More than a decade ago, a brief report raised concern about the presence of fluoxetine in aquatic systems and provided an assessment of its impact on the aquatic community (Brooks et al., 2003). Since then, a number of studies have investigated the impact of fluoxetine in fish (reviewed in Brooks, 2014; Mennigen et al., 2011; Stewart et al., 2014; Sumpter et al., 2014). Understandably, the main focus has been on their potential effects on behaviour, although possible effects on the reproductive system in fish have also been investigated. For example, Schultz et al. (2011) observed induction of plasma vitellogenin and altered testis morphology (significant interstitial cell hypertrophy) but no changes in the reproductive behaviour when male fathead minnows (*Pimephales promelas*) were exposed to 28 ng fluoxetine/L. Other studies showed elevated luteinizing hormone (LH) levels in goldfish, *Carassius auratus* (Somoza and Peter, 1991), and elevated plasma oestradiol (E_2) levels in female Japanese medaka, *Oryzias latipes* (Foran et al., 2004) and goldfish (Mennigen et al., 2008). Mennigen et al. (2010) reported that exposure to fluoxetine decreased milt volume and reduced testosterone levels while increasing E_2 levels in male goldfish. More recently, a whole issue in the journal of Aquatic Toxicology has been dedicated to antidepressants in the aquatic systems with reviews on both fish and invertebrate data (Brooks, 2014; Fong and Ford, 2014; Sumpter et al., 2014). Fluoxetine has been reported to have an impact on male fathead minnow mating behaviours and predation avoidance behaviour at 1 μg/L, as well as feeding at 10 μg/L (Weinberger and Klaper, 2014), while other antidepressants affected a variety of endpoints such as growth, reproductive fitness and predation behaviour in fish (Bisesi et al., 2014; Hedgespeth et al., 2014; Olsén et al., 2014; Yang et al., 2014).

In other vertebrates, fluoxetine treatment suppressed E_2 levels in ovariectomised rats (*Rattus norvegicus*) treated with oestrogen (Taylor et al., 2004). It reduced the number of female rats displaying oestrous behaviour (Matuszczyk et al., 1998a) and decreased sexual motivation in male rats (Matuszczyk et al., 1998b). Similar responses were observed in humans (Balon, 1995). Two studies showed that, although fluoxetine altered libido and sexual function in both rats and humans, there was no significant change in testosterone levels (Bell et al., 2006; Taylor et al., 1996). However, more recent studies have shown that fluoxetine has mild effects on hormone levels in humans, with testosterone levels being lower but only after 6 months or more of therapy (Motlagh et al., 2012). In addition, a 60-day exposure to fluoxetine (200 mg/kg body weight) resulted in adverse effects in adult male rats, such as reduced sperm motility and significant decrease in testosterone levels (Bataineh and Daradka, 2007).

Furthermore, it has also been reported that certain SSRIs can interfere with synthesis of androgens *in vitro* (Fernandes et al., 2011) via inhibition of key enzymes (C17, 20 lyases and CYP11β). If this *in vitro* activity is confirmed *in vivo* then SSRIs may be considered as pharmaceuticals with high endocrine disrupting potency.