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Environmentally relevant exposure to an antidepressant alters courtship behaviours in a songbird

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HIGHLIGHTS

- Birds eat sewage-contaminated prey containing antidepressants such as fluoxetine.
- Male starlings sang less to fluoxetine-treated females than to control females.
- Increased male aggression towards fluoxetine-treated females.
- First evidence of fluoxetine-induced courtship disruption in a songbird.
- Antidepressants in the environment alter fitness-related traits in wildlife.

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ABSTRACT

Pharmaceuticals in the environment are a recently identified global threat to wildlife, including birds. Like other human pharmaceuticals, the antidepressant fluoxetine (Prozac) enters the environment via sewage and has been detected at wastewater treatment plants. Birds foraging on invertebrates at these sites can be exposed to pharmaceuticals, although the implications of exposure are poorly understood. We conducted experiments to test whether chronic exposure to a maximally environmentally relevant concentration of fluoxetine (2.7 μg day^{-1}) altered courtship behaviour and female reproductive physiology in wild-caught starlings (*Sturnus vulgaris*), a species commonly found foraging on invertebrates at wastewater treatment plants. When paired with a female over two days, males sang less and were more aggressive towards fluoxetine-treated females than controls. Fluoxetine-treated females were initially aggressive towards males, becoming significantly less aggressive by the second day. In contrast, control females expressed intermediate levels of aggression throughout. We found no effect of female treatment on female courtship behaviour. Female body condition, circulating testosterone and circulating oestradiol were unaffected by treatment and did not account for male preference. Our findings suggest that exposure to an antidepressant reduced female attractiveness, adding to growing evidence that environmental concentrations of pharmaceuticals can alter important traits related to individual fitness and population dynamics.

1. Introduction

Chemical contaminants are a driver of global biodiversity loss, representing an additional stressor to wildlife already under pressure from factors such as habitat loss and climate change (Novacek and Cleland, 2001). In recent years, pharmaceuticals that contaminate the environment have been identified as a potential risk to wildlife, including birds (Shore et al., 2014; Arnold et al., 2014). An extreme example of this threat was demonstrated by the deaths in India of *Gyps* vultures from diclofenac residues in cattle carcasses, which led to local population collapse (Oaks et al., 2004). Direct mortality as a result of exposure to pharmaceuticals at environmental concentrations is apparently rare, yet such contaminants can instead exert sublethal effects on wildlife (Shore et al., 2014).

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Psychotropic pharmaceuticals, such as antidepressants, are designed to alter behaviour at low doses and so have the potential to modulate wildlife behaviours, with implications for individual fitness and even population persistence (Brodin et al., 2014). To date, few studies have explored the behavioural effects of exposure to psychotropic pharmaceuticals, such as antidepressants, in wild terrestrial vertebrates, including birds (Bean et al., 2014).

A widely prescribed antidepressant of the selective serotonin reuptake inhibitor class, Fluoxetine (Prozac®), has been identified as a contaminant of environmental concern (Kumar and Xagoraraki, 2010). Prescriptions of fluoxetine have been rising in the UK, increasing by 19% between 2011 and 2016 to 6.59 million items per year (HSCIC and Team, 2017). Since approximately 24% of fluoxetine is excreted as the parent compound by human patients (Lienert et al., 2007), fluoxetine has been detected at wastewater treatment plants in influent and effluent water at the ng L^{-1} level (Lajeunesse et al., 2012). However, one recent UK-based study reported a far greater concentration of 1310 ng L^{-1} in sewage influent (Bean et al., 2017). Fluoxetine has also been detected in sewage sludge at the μg kg^{-1} level (Jones et al., 2014) and treated sewage sludge is used as fertiliser on agricultural land, representing an important entry route to the terrestrial environment (Redshaw et al., 2008). Due to its high sorption coefficient, fluoxetine can persist in soils for many months (Arnold et al., 2014; Redshaw et al., 2008), during which time it can be incorporated into crops (Wu et al., 2010) and invertebrates (Carter et al., 2014). At wastewater treatment plants, birds and bats that forage directly on invertebrates at filter beds (Bean et al., 2017; Fuller and Glue, 1978) or airborne insects that spend their larval stages in wastewater tanks (Park and Cristinacce, 2006), risk exposure to comparatively high concentrations of pharmaceutical contaminants, such as fluoxetine. For example, earthworms (*Eisenia fetida*) taken from trickling filter beds at wastewater treatment plants contained up to 53.8 ng g^{-1} fluoxetine (Bean et al., 2017). Yet most studies to date have focused on aquatic ecosystems and species, whilst comparatively little research has investigated the impact of terrestrial exposure routes on free-living vertebrates, including birds (Arnold et al., 2014).

The consequences of such exposure in wild birds are also poorly understood. The evolutionarily ancient serotonergic system, including the primary target of fluoxetine (SERT), is well-conserved across vertebrates (Lillesaar, 2011). In line with the read-across hypothesis (Huggett et al., 2003), we might predict effects similar to those observed in humans in birds and mammals, following exposure to fluoxetine. Sexual dysfunction is a common side effect of fluoxetine in humans, causing delayed ejaculation in men, anorgasmia in women and decreased libido in both sexes at therapeutic dosages (typically 20–60 mg day^{-1}) (Higgins et al., 2010), with similar effects in rodents (after 10 mg kg^{-1} injected daily) (Matuszczyk et al., 1998; Uphouse et al., 2006; Sarkar et al., 2008). Fluoxetine has also been shown to increase circulating testosterone in depressed female human patients at therapeutic dosages (Kumsar et al., 2014). However, it is challenging to extrapolate to free-living vertebrates using such data, as clinical studies often employ dosages several orders of magnitude higher than environmental concentrations. In fish, reproductive behavioural and physiological responses to environmentally relevant concentrations of fluoxetine have proven highly variable between exposure concentrations, and between and within species (Sumpter et al., 2014). Effects on the frequency of certain male courtship behaviours have been observed at ~0.5 μg L^{-1} in some species (e.g. Eastern mosquitofish (*Gambusia holbrooki*) (Bertram et al., 2018)) but not others (e.g. Siamese fighting fish (*Betta splendens*) (Dzieweczynski and Hebert, 2012)), and a far lower exposure concentration (40 ng L^{-1}) has been found to increase sperm count and reduce body condition in male Eastern mosquitofish (Bertram et al., 2018). In goldfish (*Carassius auratus*), a 14-day exposure to 0.54 μg L^{-1} fluoxetine was shown to decrease circulating oestradiol in females (Mennigen et al., 2017), whilst another study found no effect of fluoxetine (water concentration range 0.1–100 μg L^{-1}) on oestradiol in female Fathead minnows (*Pimephales promelas*) exposed for 4 weeks (Weinberger and Klaper, 2014). The same study showed that fluoxetine altered male but not female mating behaviour in Fathead minnows (Weinberger and Klaper, 2014). To put these exposures into context with environmental concentrations, the median concentration in the effluent of 162 UK wastewater treatment plants was found to be 23 ng L^{-1} (5th percentile 5 ng L^{-1}, 95th percentile 69 ng L^{-1}) (Gardner et al., 2012), although concentrations ranging into the hundreds of nanograms have occasionally been reported in treated wastewater (Metcalfe et al., 2010). Therefore, the exposures in these studies can be considered to reflect worst-case exposure scenarios within the aquatic environment. Nevertheless, fluoxetine exposure has the potential to alter sexual behaviour and sex hormone levels in free-living vertebrates.

Sexual behaviour, or courtship, and sex hormones have been well studied in both free-living and captive birds (Eens et al., 1991; Pinxten et al., 2003; Dawson, 2008), albeit rarely in the context of ecotoxicology, although see (Markman et al., 2008). In songbirds, male song is known to vary according to environmental stressors such as food availability (Ritschard and Brumm, 2012) and has previously proven a sensitive endpoint for studying the effects of certain contaminants (Markman et al., 2008) and anthropogenic disturbances (Kempenaers et al., 2010). Male song is under strong sexual selection pressure and is a signal of male quality that females use to make mate choice decisions (Eens et al., 1991). However, some degree of mutual mate choice is predicted in species with biparental care (Edward and Chapman, 2011), such as the Eurasian starling (*Sturnus vulgaris*). Male starlings are selective in their mate choice and can choose females based on factors such as plumage iridescence or age (Komdeur et al., 2005). Males exercising mate choice might be expected to invest less time singing to less attractive or lower quality females; as observed in Bengalese finches (*Lonchura striata domestica*) (Heinig et al., 2014). If fluoxetine alters female reproductive behaviour or physiology in starlings and thereby alters female attractiveness, this could alter signalling of individual quality (Markman et al., 2008) by females, with associated consequences for male courtship responses and male mate choice. This could impact on individual fitness by reducing reproductive success, with predicted negative impacts at the local population level (Brodin et al., 2014).

The aim of this study was to assess whether a maximally environmentally relevant concentration of fluoxetine affected courtship behaviour or physiology in a songbird, in terms of male responses to fluoxetine-treated and control females respectively. We first investigated whether female treatment affected the following behavioural measures: a) male courtship song; b) male aggressive or courtship behaviour. We then tested whether female treatment altered female aggressive or courtship behaviour. We also determined whether treatment altered the following physiological measures in females: circulating testosterone, circulating oestradiol or body condition index. Finally, we explored whether female circulating testosterone, circulating oestradiol, body condition index, aggression or courtship behaviour accounted for variation in male behaviours.

2. Methods

2.1. Ethics statement

This work was carried out under a Home Office Licence (PPL 60/