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The antidepressants venlafaxine ("Effexor") and fluoxetine ("Prozac") produce different effects on locomotion in two species of marine snail, the oyster drill (*Urosalpinx cinerea*) and the starsnail (*Lithopoma americanum*)

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ABSTRACT

Human antidepressants have been previously shown to induce foot detachment from the substrate in aquatic snails. Prior to foot detachment, antidepressants also affect snail crawling speed. We tested two commonly prescribed antidepressants, venlafaxine ("Effexor") and fluoxetine ("Prozac") on crawling speed and time to reach the air–water interface in two species of marine snail, the oyster drill *Urosalpinx cinerea* and the American starsnail *Lithopoma americanum*. Exposure to venlafaxine increased crawling speed in both species, while fluoxetine slowed them down. Our lowest LOEC (lowest observed effect concentration) was 31.3 μg/L venlafaxine in *Urosalpinx*. Similarly, snails (*L. americanum*) exposed to venlafaxine tended to move faster and more often to the air–water interface, but exposure to fluoxetine slowed them down. Our lowest LOEC was 345 μg/L fluoxetine in *Lithopoma*. These results indicate that venlafaxine boosts locomotion, while fluoxetine reduces it, and both behaviors are preludes to foot detachment. The different effects of these two antidepressants on snail locomotion suggest differing physiological mechanisms of action in marine snails as well as possible ecological consequences.

1. Introduction

Active pharmaceutical ingredients (APIs) discharged by wastewater treatment plants are chemical contaminants of emerging concern that have been detected downstream in rivers, estuaries, and the ocean (Metcalfe, 2013). Within the last 10 years studies have documented environmental concentrations of APIs (Brausch et al., 2012; Santos et al., 2010; Kolpin et al., 2004) as well as behavioral alterations in aquatic organisms induced by APIs (Guler and Ford, 2010; Gaworecki and Klaine, 2008).

Among these APIs, antidepressants such as selective serotonin reuptake inhibitors (SSRIs) and selective serotonin/norepinephrine reuptake inhibitors (SSNRIs) have been detected in wastewater (Schultz et al., 2010; Metcalfe et al., 2010; Schultz and Furlong, 2008), in animal tissues (Brooks et al., 2005), and have been shown to modulate biological activity especially in aquatic molluscs and crustaceans (see a review by Fong and Ford, 2014).

Marine molluscs have shown particular sensitivity to antidepressants. Fluoxetine induces spawning in bivalves (Honkoop et al., 1999), larval metamorphosis in gastropod larvae (Couper and Leise, 1996) and alters cognitive abilities in cephalopods (Di Poi et al., 2013). Fong and Molnar (2013) reported foot detachment from the substrate at concentrations from 43 μg/L to 4.34 mg/L in five species marine snail from different habitats. Prior to foot detachment, the authors also observed a change in the crawling behavior of the tested species, some showing an increase in velocity. Foot detachment is a sub-lethal effect that could have lethal consequences for any aquatic snail (Fong and Molnar, 2013). Changes in crawling behavior prior to foot detachment could have different sub-lethal effects such as the inability to avoid predators and in the case of intertidal snails, altering the timing of movement to the air–water interface.

We tested the effects of two antidepressants (venlafaxine and fluoxetine) on crawling speed and ability to reach the air–water interface in two species of marine snail (*Urosalpinx cinerea* and

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Lithopoma americanum). We chose these antidepressants because both are commonly prescribed in Europe and North America (RxList, 2006) and cause foot detachment in the tested species (Fong and Molnar, 2013). Fluoxetine was ranked in the top 20 of priority endocrine disrupting chemicals for ecological effects by Kumar and Xagoraraki (2010). Furthermore, venlafaxine has been reported in wastewater effluent as high as 2.19 μg/L from Minnesota (Schultz and Furlong, 2008) and >1.0 μg/L in Canadian wastewater (Metcalfe et al., 2010). The two snail species were selected for several reasons. Both species respond to antidepressants (fluoxetine, venlafaxine, fluvoxamine) by detaching their feet from the substrate and thus their locomotion is affected. They are in different families (Muricidae and Turbinidae, respectively) with different modes of life (carnivorous predation and herbivory), from different habitats (cool temperate vs. warm temperate intertidal) and because their families include genera that have a worldwide distribution in tropical, subtropical, and temperate marine environments (Fong and Molnar, 2013).

2. Materials and methods

2.1. Species, collection sites, and maintenance

Oyster drills (*U. cinerea*, shell length 15–20 mm) were collected in December, 2013 from San Francisco Bay and immediately shipped to Gettysburg College, where they were maintained in recirculating artificial seawater (Coralife, scientific grade marine salt dissolved in deionized water, Energy Savers Unlimited, Inc., Carson, California, USA) at 30 ppt, 20° C, and fed mussels *ad libitum*. This species is well known for its hardiness in the laboratory (Morris et al., 1980). Specimens of American starsnails (*L. americanum*, shell length 10–15 mm) were collected from Key West, Florida and shipped by next day air to Gettysburg College, where they were maintained in 10 gallon aquaria with artificial seawater at 35 ppt and 25° C and supplied with thawed green algae (*Ulva* sp.). The collection sites were in areas of human habitation, but were not directly exposed to wastewater effluent. There was no mortality prior to acclimation or during any experiment.

2.2. Experimental design

To measure time to reach the air–water interface, snails were carefully removed from their holding aquaria and placed in 80 × 100 mm Pyrex dishes (one snail per dish) supplied with 225 ml of artificial seawater without aeration at 35 ppt (for *Lithopoma*) and 30 ppt (for *Urosalpinx*), (pH: 8.2, dissolved oxygen: 7.2 ppm). Snails were allowed to acclimate for 15 min at room temperature (22–23° C). Thereafter, 25 ml of antidepressant (either venlafaxine or fluoxetine) at a 10× higher concentration than the final concentration was added to each dish (controls received 25 ml of seawater). An experiment consisted of from 4 to 5 groups of snails (3–4 drug concentrations and a control). Each experiment lasted four hours, during which time, snails were monitored for frequency and crawling time to the air–water interface. The distance to the air–water interface from the bottom of each dish was 3.5 cm. A snail was counted at the air–water interface if a portion of its foot broke the water surface and the snail remained there for at least 10 min (in most cases, when a snail reached the air–water interface, it did not leave that position for the duration of the experiment).

To measure crawling speed, we tested snails before exposure to antidepressants, returned them to their respective holding aquaria for 24 h before testing them after exposure to antidepressants. This "before and after" design was employed because there is considerable between-animal variation in crawling speed. For identification, snails were marked with bee tags (queen bee marking kits, Beeworks, Orillia, Ontario, Canada) attached to the shell with a tiny drop of cyanoacrylate glue ("Krazy Glue"). We measured snail crawling speed in plastic containers (28 × 15 × 10 cm) with 1 cm^2 grids attached to the underside. Each snail was acclimated in the chamber with 900 ml of seawater for at least 20 min at room temperature. After the acclimation period, 100 ml of either seawater (for the "before drug" runs) or antidepressant at a 10× higher concentration than the final concentration, was gently added to the container. Each snail was then monitored for 20 min and the distance crawled was estimated as it moved over the grid lines.

Fluoxetine (Sigma–Aldrich, Saint Louis, MO, USA, CAS Number 56296-78-7) and Venlafaxine (AK Scientific, Union City, CA, USA, CAS Number 99300-78-4) were solubilized in artificial seawater and serially diluted. Published photodegradation data showed that none of the tested antidepressants would undergo significant photolysis in as short a time as a four-hour exposure (Kwon and Armbrust, 2006; Downing, 2004).

2.3. Statistical analyses

Except where indicated, sample sizes were $n = 12$ per drug concentration group. All concentrations were nominal and were selected because they were those concentrations and duration times used in previous experiments with these two species (Fong and Molnar, 2013). The effect of antidepressants on snail crawling speed was tested using 2-way ANOVA with repeated measures on condition (before exposure vs. after exposure) × drug concentration. For each concentration group, we calculated a mean ratio of after exposure/before exposure and used paired *t*-tests as post-hoc tests of effects of condition. Time to reach the air–water interface was tested using 1-way ANOVA with Tukey's post hoc test. Differences in the frequency of snails reaching the interface were tested with Fisher's Exact Test. Null hypotheses were rejected where $p < 0.05$.

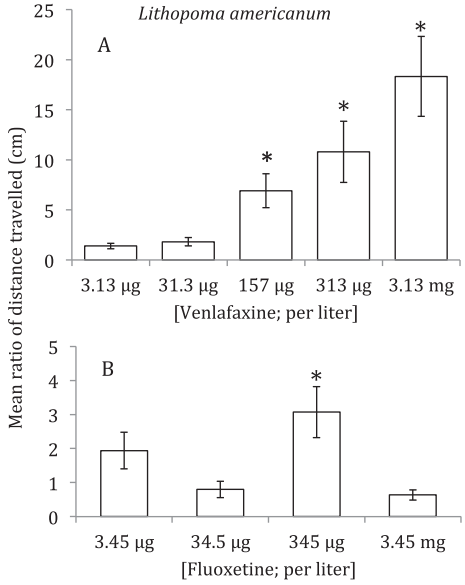

Fig. 1. Mean (±S.E.) ratio (after exposure/before exposure) of distance traveled in 20 min by *Lithopoma americanun* to different concentrations of A) venlafaxine, and B) fluoxetine. *: $p < 0.05$, paired *t*-tests of mean distance before vs. after drug exposure. Sample sizes were $n = 12$ for all groups, except for 345 μg/L fluoxetine, $n = 15$.