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Low-dose paroxetine exposure causes lifetime declines in male mouse body weight, reproduction and competitive ability as measured by the novel organismal performance assay

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ABSTRACT

Paroxetine is a selective serotonin reuptake inhibitor (SSRI) that is currently available on the market and is suspected of causing congenital malformations in babies born to mothers who take the drug during the first trimester of pregnancy. We utilized organismal performance assays (OPAs), a novel toxicity assessment method, to assess the safety of paroxetine during pregnancy in a rodent model. OPAs utilize genetically diverse wild mice (*Mus musculus*) to evaluate competitive performance between experimental and control animals as they compete among each other for limited resources in semi-natural enclosures. Performance measures included reproductive success, male competitive ability and survivorship. Paroxetine-exposed males weighed 13% less, had 44% fewer offspring, dominated 53% fewer territories and experienced a 2.5-fold increased trend in mortality, when compared with controls. Paroxetine-exposed females had 65% fewer offspring early in the study, but rebounded at later time points, presumably, because they were no longer exposed to paroxetine. In cages, paroxetine-exposed breeders took 2.3 times longer to produce their first litter and pups of both sexes experienced reduced weight when compared with controls. Low-dose paroxetine-induced health declines detected in this study that were undetected in preclinical trials with doses 2.5–8 times higher than human therapeutic doses. These data indicate that OPAs detect phenotypic adversity and provide unique information that could be useful towards safety testing during pharmaceutical development.

1. Introduction

Selective serotonin reuptake inhibitors (SSRIs) are the most commonly prescribed antidepressants and are used to treat the majority of depression cases during pregnancy (Meunier et al., 2013). Paroxetine [Paxil®, GlaxoSmithKline (GSK), Brentford, England] became commercially available in 1992 and has been used to treat patients with depression, anxiety and other mood disorders. Paroxetine, along with other SSRIs, are suspected of causing congenital cardiac defects and pulmonary hypertension when a fetus is exposed during the first trimester; however, these epidemiological studies are controversial because some studies find correlative evidence (Williams and Wooltorton, 2005; Bérard et al., 2007; Diav-Citrin et al., 2008; Ellfolk and Malm, 2010) and others do not (Kulin et al., 1998; Källén and Otterblad Olausson, 2007). Despite this controversy, in 2005, the Food and Drug Administration (FDA) requested that paroxetine be labeled as a class D drug (positive evidence of human fetal risk) and issued a warning that paroxetine exposure in the first trimester may potentially cause birth defects (FDA, 2005).

The preclinical assessment of paroxetine followed the typical protocol in which the drug was tested to determine whether it was mutagenic, carcinogenic, teratogenic or if it caused infertility (GSK, 2013). No genotoxic effects were detected in rodent cells, and tumors were detected in mice and rats at doses 2-3.9 times the maximum recommended human dose (GSK, 2013). Teratogenicity was not assessed in mice and no teratogenic effects were in observed in rats when given a dose >8 times higher than human therapeutic doses (GSK, 2013). Paroxetine effects on fertility were not assessed in mice, but the drug impaired fertility in rats at high doses; that is, when females were given doses 2.5-fold higher and when males were given >8 times higher than human therapeutic doses (GSK, 2013). Another traditional assay that is evaluated during preclinical trials is the functional observational battery (FOB). FOBs consists of several important behavioral assays and autonomic tests to evaluate whether a substance causes neurotoxicity (Moser, 2011), however, no data from such tests are reported for paroxetine exposure (GSK, 2013). After successful completion of preclinical assessment, paroxetine was deemed safe and continued onto clinical trials until it was released onto the market.

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Like paroxetine, many medications once considered safe are found to cause unacceptable health consequences after public release. On average, 73% of pharmaceuticals fail during clinical trials (Lipsky and Sharp, 2001) and 10% of FDA approved pharmaceuticals are recalled after market release (Schuster et al., 2005), despite the 12–15 years of research and $1.4 billion average cost associated with each drug during development (Miller, 2012). One cause of the high pharmaceutical failure rate is the inability of current toxicity assessment methods to detect cryptic, or otherwise undetectable, adversities during preclinical trials, particularly those present at doses near therapeutic levels and/or those occurring at low incidences.

We have developed a novel toxicity assessment research method that may be useful during preclinical assessment, known as the organismal performance assay (OPA). In several instances, OPAs have proven capable of detecting mammalian health declines that were not visible to standard laboratory methodologies. OPAs utilize genetically diverse wild-derived mice (*Mus musculus*) that compete among each other for limited resources in semi-natural enclosures, which allows for direct competition between treatment and control individuals. OPAs assess the quality of individual mice (organisms) in terms of Darwinian fitness (i.e., reproductive success) and components leading to fitness (i.e., survivorship and male competitive ability), while residing in a naturalistic environment where the stresses that have shaped their evolutionary history are present. The sensitivity of the OPA derives from the fact that wild mice under social competition allows small changes in behavior or physiological performance and otherwise cryptic effects of toxicity to be manifested as measureable negative outcomes; such as relegation to inferior habitat and reduced reproduction and survival. Consequently, any degradation in almost any physiological system caused by a treatment will be detectable by the inability of mice to perform comparable to controls with whom they compete and will be revealed in OPA endpoint measures. OPAs have previously been used to quantify the adverse effects of sibling-level and cousin-level inbreeding (Meagher et al., 2000; Ilmonen et al., 2008), harboring a selfish gene (Carroll et al., 2004) and recently, they were the first assay to reveal the adverse effects of added sugar consumption at human-relevant levels (Ruff et al., 2013). In all of these studies, OPAs found substantial deleterious effects that were missed by current methodologies.

Here OPAs are used to determine if paroxetine exposure near human therapeutic doses during in utero and into early adulthood cause fitness declines in wild mice. If paroxetine exposure adversely affects any physiological system, we hypothesize that exposed individuals will suffer reproduction and survival declines relative to control individuals while competing within enclosures. Furthermore, while generating animals for OPAs, we assessed whether paroxetine exposure negatively affects reproduction of exposed breeders and the weight of the resulting offspring.

2. Materials and methods

2.1. Animals

Wild-derived outbred house mice were used in this experiment. Unlike many genetically inbred mouse strains, wild mice have behavioral characteristics that allow them to function in natural and semi-natural environments (Nelson et al., 2013). In this experiment, individuals were from the 12th generation of the colony described by Meagher et al. (2000). Genetic diversity of this colony was assessed in the 11th generation and found to be comparable to wild populations (Cunningham et al., 2013). Within enclosures and breeding cages, individuals were provided access to food and water ad libitum and maintained on a 12:12 hour light:dark cycle. All procedures were approved by the University of Utah IACUC.

2.2. Drug exposure

Dosing was achieved by incorporating 7.5 g paroxetine (GSK, molecular formula: $C_{19}H_{20}FNO_3 \cdot HCl$) into 50 kg of rodent chow (TD.130006; Harlan Teklad, Madison, WI). Mice consuming an average of 3 g of food per day and weigh 20 g will ingest 0.45 mg per day or 22.5 mg/kg/day. Using a standard metabolic rate conversion factor, this is equivalent to a human dose of 1.82 mg/kg/day, or a daily dose of 109.20 mg, assuming the average human weighs 60 kg (Reagan-Shaw et al., 2008). Given that paroxetine is prescribed in the range of 20–60 mg/day (Dunner and Dunbar, 1992; GSK, 2013), our dose is 1.82-fold higher than human therapeutic doses, yet lower than doses used in previous animal studies (Coleman et al., 1999; Rayburn et al., 2000). Although we did not determine serum levels, one study determined that a paroxetine dose of 30 mg/kg/day achieved serum levels in mice that were comparable to human serum levels when taking the highest therapeutic dose (Coleman et al., 1999).

Sixty breeder pairs were selected for this experiment; 20 pairs were exposed to paroxetine while the remainder served as controls. The asymmetry in cage number is due to the production of additional control animals for another study. Prior to breeding, animals were individually housed and provided with their respective diets. To maximize the chances of detecting adverse effects, both females and males were exposed to paroxetine prior to breeding (females were exposed to paroxetine eight days prior and males five days). Exposure to paroxetine continued when breeders were paired. By exposing both females and males, we were consistent with previous rodent studies (Coleman et al., 1999; Rayburn et al., 2000; El-gaafarawi et al., 2005), and it is likely that any adverse effects detected in the progeny are due to in utero exposure because birth defects have been observed in humans when women are prescribed paroxetine during pregnancy (Diav-Citrin et al., 2008). Breeding pairs were kept together until a maximum of four litters were produced to ensure enough animals for OPA assessment. At 28 days of age, pups were weaned and housed in same-sex sibling cages. Upon weaning, individual weight, sex and litter size data were collected and paroxetine exposure continued until animals were released into semi-natural enclosures (Fig. 1). By exposing offspring in utero and through early adulthood (rather than stopping the exposure at weaning), the duration maximized the ability of OPAs to detect health consequences. This is because once animals were released into enclosures they were all fed the control diet, as currently, we are unable to keep animals on their respective diets while they are free ranging during OPAs. Upon release into enclosures, both paroxetine-exposed and control animals were provided with the control diet ad libitum; switching the paroxetine-exposed animals to the control diet (rather than switching control animals to the paroxetine diet) was a more conservative approach of detecting fitness impacts, because OPAs would then be assessing the effects of a previous exposure.

2.3. Semi-natural enclosures

Enclosures have previously been described in Ruff et al. (2013). Briefly, the indoor enclosures are approximately 30 m^2 and consist of two types of territories, optimal ($n = 4$) and suboptimal ($n = 2$). Each optimal territory contained a defendable box with multiple dark nesting sites and direct access to food. Suboptimal territories contained two nesting boxes exposed to light and had indirect access to food. Territories were separated by hardware mesh that is easily climbed, but added an element of spatial complexity (Fig. S1).

Five independent OPA populations were established and maintained for 28 weeks. OPA populations consisted of eight males and 14-16 females, for a total of 116 animals (40 males, 76 females); these animals are referred to as population founders. Half of the individuals of each sex were paroxetine-exposed while the remainder served as controls; this population structure allows paroxetine individuals to directly compete