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Male rats with same sex preference show high experimental anxiety and lack of anxiogenic-like effect of fluoxetine in the plus maze test

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ABSTRACT

Homosexual men show a 2–4 higher risk to suffer anxiety in comparison with heterosexuals. It is unknown if biological factors collaborate to increase such incidence. Fluoxetine produces differential brain activation in homosexuals as compared with heterosexuals, suggesting that it may produce a divergent behavioral effect dependant on sex-preference. The first aim was to evaluate experimental anxiety in male rats that show same-sex preference in the elevated plus maze (EPM). The second goal explored the putative differential effect of fluoxetine (10 mg/kg) in male rats with female and same-sex preference in the EPM. To induce same-sex preference males were prenatally treated with letrozole (0.56 μg/kg, 10–20 gestation days), while controls were males prenatally treated with letrozole that retain female-preference or which mothers received oil. In both groups we found animals with male preference, but the proportion was higher in males that prenatally received letrozole (10 vs. 27%). Males with same-sex preference spent less time and showed lower number of entries to the open arms of the EPM than males that prefer females, regardless of the prenatal treatment. In males with female preference, fluoxetine reduced the time spent and number of entries to the open arms that was absent in males with same-sex preference. These data suggest that biological factors contribute to the high levels of anxiety in subjects with same-sex preference and that fluoxetine in men may produce a divergent action depending on sexual orientation.

1. Introduction

Pathological anxiety (any anxiety disorder) has a much higher prevalence (41%) in homosexual men as compared with its heterosexual counterpart (18%) (Bostwick et al., 2010; Herek and Garnets, 2007; Sandfort et al., 2001). Such different prevalence is nearly four times in panic disorder with agoraphobia (Bostwick et al., 2010). The reasons underlying this increased prevalence primarily rely in social factors that include discrimination, stigmatization and harassment (Meyer, 2003). To our knowledge, no study has analyzed the putative biological factors that may contribute. Thus, the first part of this study analyzes whether the experimental anxiety levels differ between male rats with same-sex preference and those with female-preference. We hypothesized that males with same-sex preference would exhibit higher experimental anxiety. Experimental anxiety was assessed in the elevated plus maze (EPM), which partially model the panic with agoraphobia disorder (Lister, 1990; Pinheiro et al., 2007; Carobrez and Bertoglio, 2005).

The pharmacological treatment of pathological anxiety comprises mainly three drug groups: benzodiazepines, monoamine reuptake inhibitors and pregabalin (Baldwin et al., 2011, 2014). A recent meta-analysis (Baldwin et al., 2011) compared the effectiveness and tolerance of these treatments for generalized anxiety disorder. These authors found that the most effective drug was fluoxetine in comparison with other selective serotonin reuptake inhibitors (SSRI) including escitalopram, paroxetine and sertraline, or dual noradrenalin–serotonin reuptake inhibitors such as duloxetine, or even than benzodiazepines.

Fluoxetine behavioral actions have been well characterized in various animal models. In particular in animal models of obsessive compulsive disorder (OCD) (Fernandez-Guasti et al., 2006; Kobayashi et al., 2008; Olvera-Hernandez et al., 2013; Umathe et al., 2011), in some features of major depression (Fitzgerald and Bronstein, 2013; Page et al., 1999; Recamier-Carballo et al., 2012) and in paradigms established to study experimental anxiety. Regarding the latter, fluoxetine has yielded controversial results in the elevated plus maze (EPM) test, where some authors have reported anxiolytic-like actions (Griebel et al., 1999; Rogoz and Skuza, 2011) while others found anxiogenic-like effects (Robert et al., 2011; Silva et al., 1999; Silva and Brandao, 2000) or even lack of results (Borsini et al., 2002; Griebel et al., 1997, 1999; Pinheiro et al., 2007; Silva and Brandao, 2000), using similar doses (5 and 10 mg/kg). The reasons underlying these

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differences involve administration schedule (chronic vs. acute), animal's strain, illumination intensity and manipulation during the test (Robert et al., 2011).

Kinnunen et al. in, 2004 reported for the first time the relation between sexual orientation and cerebral function through positron emission tomography (PET) after administering a fluoxetine's challenge. They found glucose consumption differences between homosexual – Kinsey 6 subjects – and heterosexual men in various brain areas. Interestingly, the hypothalamus of homosexual men showed a smaller activation in comparison with that of heterosexuals, while in the prefrontal cortex there was a lower metabolism in homosexuals remaining without change in heterosexuals. These data suggest that fluoxetine could have divergent behavioral actions between homosexual and heterosexual men. Thus, the second aim of the present study was to analyze putative dissimilar effects of fluoxetine in the EPM of male rats with male- or female-sex preference.

Following previous reports (Olvera-Hernandez et al., 2015; Olvera-Hernandez and Fernandez-Guasti, 2015) we used male rats with same-sex preference which mothers were injected with letrozole, a third generation aromatase inhibitor (Dutta and Pant, 2008), for 10 days (G10–20) at a dose of 0.56 μg/kg. As controls we used males of the same litter which mothers received letrozole, but that retain clear female preference. To explore if differences were due to prenatal letrozole treatment we also included males which mothers received oil. To discern selective fluoxetine's effects we analyzed its effects on motor activity and coordination.

Sex preference is usually based on the amount of time the subject spends in either the male or the female compartment in choice paradigms (Avitsur and Yirmiya, 1999; Everitt, 1990; Vasey, 2002). Previous data (Olvera-Hernandez et al., 2015; Olvera-Hernandez and Fernandez-Guasti, 2015) revealed that prenatal treatment with letrozole increased the time that some males spend in the male's chamber. Such increase was unaccompanied by changes in the time spent in the neutral compartment (without stimulus animal). In addition, a considerable proportion of males that preferred to stay in the male's vicinity also showed higher interaction, female sexual behavior (lordosis and proceptivity) and non-contact penile erections when exposed to sexually experienced males, suggesting that the male-preference (produced by letrozole) is not due to reduced social behavior or low sexual interest. In addition, no difference was found in the serum levels of testosterone, estradiol, LH and FSH between control and letrozole-treated males regardless of their sexual preference (Olvera-Hernandez et al., 2015).

2. Materials and methods

2.1. Animals

Female and male Wistar rats were used in this study. They were housed in a room with controlled temperature (22 ± 2 °C) conditions under an inverted 12 hour light–dark cycle (lights off at 10:00 h); with *ad libitum* access to water and food throughout the experiments. All procedures were performed in accordance with the Mexican Official Norm for the use and care of laboratory animals "NOM-062-ZOO-1999" and NIH publication 85–23, 1985 and approved by the local Ethics Committee (CICUAL-Cinvestav).

2.2. Letrozole treatment

The method of letrozole administration has been previously described in detail (Olvera-Hernandez et al., 2015). Briefly, females in proestrus were mated with a sexually experienced male until receiving two or three ejaculations and parturition occurred 22–23 days later. Treatment consisted of a daily subcutaneous injection of letrozole (Sigma-Aldrich, St. Louis, USA) at the dose of 0.56 μg/kg/ml (dissolved in corn oil) or vehicle to the mothers from day 10 of pregnancy until one day before delivery. The day of birth the pups were culled to four males and four females. The pups were weaned at 21 days of age and housed 6–8 males with the same treatment per cage.

2.3. Behavioral observations

All observations were started when the males reached the age of three months and weighed between 280 and 340 g. All tests were begun at 11:00 h in a room under dim red light (11.5 lx). Partner preference and experimental anxiety tests were videotaped for later analyses.

All animals were tested for sex-preference and divided into two groups: males with preference for other males and males with female-preference. Three days later the animals experienced the EPM test. The number of animals per group is indicated in figures (number in parenthesis under each bar).

2.3.1. Three-compartment partner preference test

This test was previously described by Brand et al. (1991) and Olvera-Hernandez et al. (2015). Briefly, the apparatus consisted of a box with 3 compartments (60 × 30 × 40 cm each) and a small opening (13 × 12 cm) in both partitions near the front. The left and right compartments contained stimulus animals restrained with a harness in a way that they had a limited action radius but were able to display sexual behavior. Animals that served as stimuli were ovariectomized sexually receptive females and sexually experienced males. Receptivity was induced by 10 μg/rat of estradiol benzoate (Sigma-Aldrich) followed 24 h later by 2 mg/rat of progesterone (Sigma-Aldrich) that was administered 4–6 h before the test. Sexually experienced males were selected as the other stimulus. Males that achieved one ejaculation in less than 15 min in at least three of five copulatory sessions were considered as sexually experienced. During the test the experimental subjects were placed in the middle section and the time spent in each compartment recorded during 15 min. Experimental males could freely move around and interact with the stimuli. We considered a male to be in a compartment when all four limbs had entered it. A partner preference score was calculated by subtracting the time spent with the sexually active male from the time spent with the sexually receptive female. A positive score indicated preference for the female whereas a negative score revealed preference for the sexually active male (Olvera-Hernandez et al., 2015).

2.3.2. Elevated plus maze

This model consists of an elevated (40 cm above the floor) plus shaped maze with two (opposite) enclosed and two open arms, each measuring 50 cm long × 10 cm wide. The enclosed arms are surrounded by 40 cm walls (closed arms), while the other arms lacked walls (open arms). Each animal was located in the center of the maze facing a closed arm. Entry into an arm was defined as the animal placing all four paws into it. The cumulative time spent in the open arms, the number of open-arm entries and the number of total crossings was recorded over a 10 min session. The maze was cleaned with a 5% ethanol/water solution and dried thoroughly between test sessions. A decrease in the time spent and number of entries into the open arms is interpreted as an anxiogenic-like response (Pellow et al., 1985).

2.4. Experimental design

2.4.1. Experiment 1. Changes in experimental anxiety according to sex-preference

In the first part we analyzed in a very large number of subjects (n= 159) the proportion of males and the time they spent with each stimulus in the sex-preference test after prenatal treatment with oil (n=51) or letrozole (0.56 μg/kg/ml) during gestation days 10 to 20 (n=108). In the next part we selected some animals to study the putative differences in experimental anxiety in the EPM according to sex preference. These males were tested in the EPM without injection.