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Sex and age differences in the antidepressant-like effect of fluoxetine in the forced swim test

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ARTICLE INFO

Received 5 November 2015
Received in revised form 19 January 2016
Accepted 21 January 2016
Available online 22 January 2016

Keywords:
Fluoxetine
Depressive-like behavior
Forced swim test
Sex and age differences

ABSTRACT

This study compared in males and females of three representative ages: young adults (3–5 months old), middle-aged (12–15 months old) and senescent (23–25 months old) the antidepressant-like effect of fluoxetine (FLX, 5.0 and 10 mg/kg) in the forced swim test (FST). Intact (non gonadectomized) rats were evaluated. Young adult females were chosen in proestrus/estrus or in metestrus/diestrus, while middle-aged and senescent females were selected in metestrus/diestrus. Locomotion and motor coordination were also recorded. Under basal conditions (without FLX), young adult and middle-aged females showed less immobility than males. This sex difference disappeared at senescence because males diminished their levels of immobility. Thus, senescent males showed lower immobility than middle-aged and young males. FLX (5 and 10 mg/kg) produced similar actions in young females irrespective of their estrous cycle phase, therefore, these subgroups were pooled in a single one. Young adult and middle aged females clearly responded to 5 and 10 mg/kg of FLX with a reduction in immobility, while young adult and middle-aged males only did to 10 mg/kg. In senescent females 10 mg/kg FLX reduced immobility. Remarkably, in senescent males this FLX dose did not produce an antidepressant-like effect. FLX marginally affected locomotion; however, at its highest dose (10 mg/kg), and only in senescent males, interfered with motor coordination tested in the rotarod. These data show that sex and aging influence behavioral despair without treatment and after FLX.

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1. Introduction

Depression is an affective disorder characterized by chronic low mood and loss of interest in most activities as core symptoms (American Psychiatric Association, 2000). Sex and age importantly affect depression (Afifi, 2007; Blazer and Hybels, 2005). Thus, this mental illness is commonly found in the elderly, with a prevalence ranging from 22 to 46% in people over 65 years old (Lebowitz et al., 1997) and in this population becomes a heterogeneous disorder with complex genetic background (Pitychoutis et al., 2013). In addition, clinical studies have constantly observed gender differences among patients with depression, with young women outnumbering men at a rate of 2:1, a prevalence that may exacerbate to 5:1 in perimenopause (Borrow and Cameron, 2014; Cohen et al., 2003; Soares et al., 2001). Another contributing factor for the differences in depression prevalence across a woman's lifetime is changes in levels of gonadal hormones (Freeman et al., 2006; Freeman et al., 2004). Not merely the prevalence of depression varies with age and sex but also the disease symptom profile (American Medical Association Council on Scientific Affairs, 2006; Goodwin and Gotlib, 2004; Nolen-Hoeksema et al., 1999; Silverstein, 2002).

Incredibly, the efficacy/effectiveness of antidepressants in old patients has not been systematically assessed. The first line pharmacotherapy for this population is the selective serotonin reuptake inhibitors (SSRIs) (American Psychiatric Association, 2010; Coupland et al., 2011; Rajji et al., 2008) as fluoxetine (FLX), citalopram, sertraline and paroxetine (Blazer, 2003; Nelson et al., 2008). Although SSRIs are reported to be effective in clinical trials with older patients (Blazer, 2003; Gareri et al., 2000; Salzman et al., 2002), the careful literature review reveals highly variable results (Kasper et al., 2005; Nelson et al., 2008) (see Discussion). Moreover, it has been suggested that the decline in antidepressants' actions begins at middle age (Tedeschini et al., 2011), but no studies have been carried out exploring putative sex differences. The SSRIs are relatively secure compounds that have some side effects such as nausea, diarrhea and sexual dysfunction (Gareri et al., 2000; Owens et al., 1997) that unfortunately are increased in aged patients (Coupland et al., 2011).

Although controversial (Estrada-Camarena et al., 2011a; Hildebrandt et al., 2003; Lewis-Hall et al., 1997), various studies have suggested that women respond and tolerate better SSRIs than men; while men respond better to imipramine (a tricyclic antidepressant) (Kornstein et al., 2000;

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http://dx.doi.org/10.1016/j.pbb.2016.01.011
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Thase et al., 1996). In line, other authors (Berlanga and Flores-Ramos, 2006; Martenyi et al., 2001) found that women show a better response to SSRIs (FLX or citalopram) than to selective noradrenaline reuptake inhibitors (SNRI), while men respond likewise to both inhibitors. In addition to sex differences, menopausal status has also been suggested as a factor influencing antidepressants effects (Kornstein et al., 2000; Schneider et al., 1997), inviting to test these compounds in middle-aged subjects.

The most used animal model to study the antidepressant-like effect of drugs and non-pharmacological treatments is the forced swim test (FST). In it we and others have found higher immobility scores (interpreted as despair in the FST) in male than in female rats, suggesting a decreased motivation of males to escape from the stressing situation (Alonso et al., 1991; Contreras et al., 1995; Gomez et al., 2014). The lower immobility in females is inversely related to the levels of estrogen and progesterone (Barros and Ferigolo, 1998; Estrada-Camarena et al., 2011a), indicating that the FST is sensitive to steroid hormones (Andrade et al., 2010; Contreras et al., 1998; Estrada-Camarena et al., 2010; Galea et al., 2001; Rodriguez-Landa et al., 2009; Walf and Frye, 2005, 2007; Walf et al., 2004). In relation with age we and others agree that middle aged animals are more susceptible to develop depressive-like behaviors in this test (de Chaves et al., 2009; Recamier-Carballo et al., 2012).

Surprisingly, the experimental analysis of putative changes in antidepressant-like actions considering sex and age differences is scant [see the excellent review by (Kokras et al., 2015)]. In males, Bourin and colleagues found that some antidepressants were less effective in 40-week-old mice compared with 4-week-old subjects (Bourin et al., 1998; David et al., 2001a). In another study, middle-aged male rats (12–15 months old) required a longer treatment than young adults (3–5 months old) to show the antidepressant-like effect of citalopram (10 mg/kg/day) (Herrera-Perez et al., 2010). These data suggested that middle-aged males were less responsive to the antidepressant-like effects. A recent report from our group systematically analyzed this proposition and found that young adult males were sensitive to the antidepressant-like effect of FLX, at 10 mg/kg in the FST, while senescent males (23–25 months old) were completely insensitive to the antidepressant-like effects of this SSRI at 5, 10 and even 20 mg/kg (Olivares-Nazario et al., 2015). In females FLX (10 mg/kg) produced an analogous response in young adult and middle-aged ovariectomized subjects in the FST, although the young ones seem slightly more sensitive, because they showed a reduced immobility at 2.5 mg/kg that was absent in middle-aged females (Recamier-Carballo et al., 2012). These data, taken together, support the hypothesis of a lower sensitivity to antidepressants with age, particularly in males. It is also worth mentioning that working with females has the extra consideration of the estrous cycle phase.

To reduce the number of subjects, researchers have abused of ovariectomized females to explore sex differences, using intact (non orchidectomized) males. Such comparison has the flaw that one group lacks ovarian steroids while in the other there is a testicular androgen and estrogen production, which importantly modify the antidepressants action (Gomez et al., 2014; Martinez-Mota et al., 2008; Martinez-Mota and Fernandez-Guasti, 2004). Another important issue regards the different age concepts. Although it is impossible to analogously establish age correlations between rats and humans, most studies using aged rats include animals of around one year. The lifespan of laboratory rodents is about 24–34 months (Nadon, 2006), and it is quite difficult and expensive to maintain senescent animals. However, this information is essential in depression studies evaluating sex differences. On these bases the purpose of this study was to compare in males and females of three representative ages: young adults (3–5 months old), middle-aged (12–15 months old) and senescent (23–25 months old) the antidepressant-like effect of FLX in the FST. Intact (non gonadectomized) rats were used. The groups of females were selected according to their vaginal smear cytology: young adult rats were chosen in proestrus/estrus and in metestrus/diestrus, while middle-aged and senescent females were selected in metestrus/diestrus.

2. Materials and methods

2.1. Animals

Wistar male and female rats born in the local vivarium were used in this study. Animals were housed under an inverted 12-h light/12-h dark cycle (lights off at 10:00 h) in a room with constant temperature of 24 ± 1 °C. Rats had *ad libitum* access to water and commercial food during the complete study and were maintained in accordance with the Official Norm of Technical Specification for the Production, Care and Use of Laboratory Animals (NOM-062-ZOO-1999). All protocols were approved by the local committee of ethics on animal experimentation. We certify that all procedures were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23 revised 1996) and that all efforts were made to minimize the number of animals and their suffering.

2.2. Forced swim test

Swimming sessions were conducted by placing rats in individual glass cylinders (46 cm height; 20 cm diameter) containing water at 23–25 °C, 30 cm deep, so the rats could not support themselves by touching the bottom. For males, an initial 15-min session (termed pre-test) was followed 24 h later by a 5-min session (termed test). In our experience (unpublished data) the FST alters the estrous cycle in around 30% of the subjects. In view of that, in the present experiments with females the pre-test was made one week before the test (Vega-Rivera et al., 2013). The impact of the pretest on expression of despair in the test is maintained up to three weeks (Detke et al., 1997). Following each swim session, the rats were removed from the cylinders, dried with paper towels and placed into heated cages for 30 min, and then returned to their home cages. Test sessions were run between 1200 and 1500 h and videotaped for later scoring. A single observer, who was unaware of the treatments, did all the behavioral scoring. A time sampling technique was employed to score three different behaviors (Detke et al., 1995). During the test session, the scorer rated at the end of each 5-s period the following behaviors: (1) immobility—floating without struggling, and doing only those movements necessary to keep the head above the water; (2) swimming—showing active swimming motions, more than those necessary to merely keep the head above water, i.e., moving around in the cylinder or diving; and (3) climbing—presenting active movements with the forepaws, usually directed against the walls (Estrada-Camarena et al., 2003; Martinez-Mota et al., 2008).

2.3. Experimental design

Rats of different ages were randomly assigned to receive vehicle (saline solution 0.9%), 5.0 or 10 mg/kg of fluoxetine hydrochloride (Bioquimed, Mexico City, Mexico, dissolved in saline solution). These treatments were applied following a sub-acute schedule, i.e., three s.c. injections administered between pre-test and test sessions (24 h, 5 h, and 1 h before the test). The number of subjects per group is shown in Table 1. FLX was injected in a volume of 2 mL/kg and doses (expressed in terms of salt) were those given in each individual injection. FLX doses and latencies were selected from previous reports (Estrada-Camarena et al., 2003, 2004; Gomez et al., 2014; Martinez-Mota et al., 2008; Martinez-Mota and Fernandez-Guasti, 2004). Only young adult females with regular 4–5 days estrous cycles were selected. They were tested in proestrus/estrus or metestrus/diestrus, while middle aged and senescent rats were chosen in metestrus/diestrus for the 5-min session of the FST. The few aged female rats with constant estrous were discarded. Due to the shortage of senescent animals in this study we include only

Table 1
Effect of fluoxetine (5 and 10 mg/kg) on ambulation in young adult, middle-aged and senescent female and male rats.

Treatment	Young adults		Middle-aged		Senescent	
	Female	Male	Female	Male	Female	Male
Saline	n = 21	n = 9	n = 10	n = 10	n = 11	n = 14
	144.9 ± 16.8	164.7 ± 10.4	116.0 ± 20.3	117.9 ± 11.4	145.0 ± 17.6	96.8 ± 8.1[***]
FLX 5 mg/kg	n = 14	n = 7	n = 10	n = 11		
	89.9 ± 10.2[*]	128.7 ± 25.0	116.9 ± 13.1	91.4 ± 9.4		
FLX 10 mg/kg	n = 10	n = 6	n = 9	n = 12	n = 9	n = 8
	89.4 ± 6.50[**]	77.5 ± 16.8[**]	76.7 ± 8.7	109.5 ± 11.9[&]	139.9 ± 24.2	90.0 ± 12.4

Table shows mean number of counts ± S.E. Holm–Sidak post hoc test.
  [*] p < 0.05.
 [**] p < 0.01 versus saline same age and sex.
  [&] p < 0.05 versus females of the same age.

data of the group that received the largest dose of FLX (10 mg/kg). In addition to the FST, locomotor activity and motor coordination were assessed by an open-field and the rota-rod tests, performed 10 and 5 min, respectively, before the FST test session. The complete sequence of behavioral tests is shown in Fig. 1.

2.4. Open-field test

Rats were placed individually in an actimeter (45 × 45 × 20 cm, Panlab 8811-IR; Panlab, Barcelona, Spain), which registered their spontaneous ambulatory activity by infrared sensors attached to a data transfer software (Sedacom; Panlab, Barcelona, Spain). The field was wiped with a cleaning solution and completely dried before use. Results are expressed as the mean ± SEM of ambulatory movements during the test.

2.5. Rota-rod test

Each rat was placed on a rotating cylinder (7 cm diameter, 11 rpm) and its capacity to keep walking for 5 min was registered. Previously, they were trained for three consecutive days, one session daily (10, 5, and 5 min, respectively) (Fernandez-Guasti and Lopez-Rubalcava, 1998). When a rat dropped from the cylinder it was placed again; however, when it fell five times in a minute or did not show intentional movements to walk, the session was stopped and the training was continued the next day. If these conditions occurred on the testing day, the rat was judged incapable of performing the test. For each group we obtained the median number of falls and the percentage of rats able to perform the test.

2.6. Statistical analyses

The levels of immobility, swimming and climbing were analyzed by a three-way analysis of variance (ANOVA) considering sex, age and pharmacological treatment with FLX at 10 mg/kg as factors, followed

Fig. 1. Sequence of behavioral tests. FLX = Fluoxetine; VEH = vehicle; FST = forced swim test.

by two way ANOVAs within each age considering sex and FLX treatment (5 and 10 mg/kg). The putative differential effect of FLX in the two estrous cycle phases of young female rats was explored by a two way ANOVA considering phase and saline/FLX treatment. The ambulatory data and the number of falls from the rotarod were analyzed by a one way ANOVA. Pos hoc tests were made with the Holm–Sidak method. Proportions were compared with the Fisher F test.

3. Results

3.1. Sex and age differences in basal levels of immobility, swimming and climbing

The observation that young adult females in proestrus/estrus showed slightly lower levels of immobility than females in metestrus/diestrus (mean 27.3 and 28.3 number of counts, respectively) is in agreement with previous findings (Estrada-Camarena et al., 2010; Frye and Walf, 2002; Marvan et al., 1997; Schneider and Popik, 2007; Zimmerberg et al., 2005). This fase difference, however, was not statistically significant (two way ANOVA, $F_{1,47} = 1.69$, NS). Under basal conditions (without pharmacological treatment) females showed less immobility than males (three way ANOVA, sex, age and treatment interaction, $F_{2,133} = 5.495$, p < 0.01), particularly young adults (Holm–Sidak, p < 0.001, Fig. 2, left) and middle aged (Holm–Sidak, p < 0.001, Fig. 3, left). This sex difference disappeared at senescence (Fig. 4, left), because males diminished their levels of immobility (Holm–Sidak, NS). Within males treated with saline there was a reduced immobility associated with aging (three way ANOVA, age and treatment interaction: $F_{2,133} = 4.79$, p < 0.01). Thus, senescent males showed lower immobility than middle-aged (Holm–Sidak, p < 0.01) and young ones (Holm–Sidak, p < 0.01); while in females immobility slightly increase along aging without statistical significance. Females of all ages showed more swimming than males (three way ANOVA, for factor sex: $F_{1,133} = 96.35$, p < 0.001, Figs. 2–4, centre), and only senescent males showed higher levels of climbing than females (Holm–Sidak, p < 0.05, Fig. 4, right).

3.2. Effect of fluoxetine in the FST in young adults, middle aged and senescent male and female rats

The two way ANOVA considering the estrous cycle phase and the treatment with FLX (5 and 10 mg/kg) revealed that this antidepressant produced similar actions in young females chosen in proestrus/estrus than in metestrus/diestrus (immobility: estrous cycle phase, $F_{1,47} = 1.69$, NS; FLX treatment, $F_{2,47} = 14.09$, p < 0.001 and interaction $F_{2,47} = 0.34$, NS; swimming: estrous cycle phase, $F_{1,47} = 0.05$, NS; FLX treatment, $F_{2,47} = 5.67$, p < 0.01 and interaction $F_{2,47} = 0.69$, NS, and climbing: estrous cycle phase, $F_{1,47} = 0.56$, NS; FLX treatment, $F_{2,47} = 0.38$, NS and interaction $F_{2,47} = 2.55$, NS). On these bases we decided to pool both young adult female subgroups and treat them as a single one.
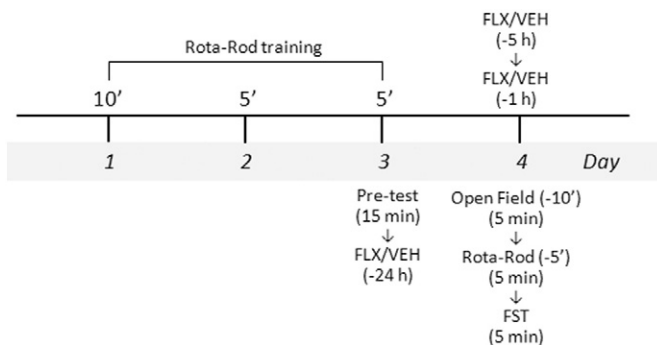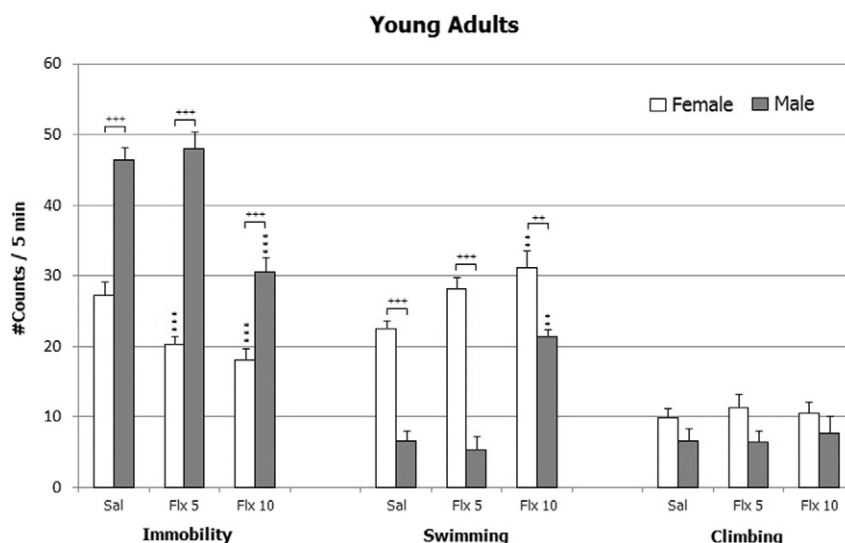
Young Adults

Fig. 2. Sex different antidepressant-like effect of fluoxetine (5 and 10 mg/kg) in young adult rats (3–5 months old) in the forced swim test. For ANOVA results see text. Asterisks over columns represent differences versus control saline treated animals, **p < 0.01; ***p < 0.001. Sex differences are shown by brackets, $^{++}$p < 0.01; $^{+++}$p < 0.001. Holm–Sidak post hoc test.

Considering the three age groups, both sexes and treatment with the highest FLX dose of 10 mg/kg, the three way ANOVA revealed a significant interaction between these factors for immobility ($F_{2,133} = 5.495$, p < 0.01) and climbing, ($F_{2,133} = 5.224$, p < 0.01), but not for swimming ($F_{2,133} = 0.731$, NS).

Young adult females clearly responded to 5 and 10 mg/kg of FLX with a reduction in immobility, while young adult males only did to 10 mg/kg (Fig. 2, left). Thus, the two way ANOVA showed a significant effect for the factor sex ($F_{1,69} = 181.12$, p < 0.001), FLX treatment ($F_{2,69} = 27.26$, p < 0.001) and an interaction between both factors ($F_{2,69} = 8.29$, p < 0.001). In both sexes the reduced immobility was accompanied by an increased swimming (sex: $F_{1,69} = 82.43$, p < 0.001; FLX treatment: $F_{2,69} = 16.32$, p < 0.001 and interaction: $F_{2,69} = 4.14$, p < 0.05) (Fig. 2, centre) and no changes in climbing (sex: $F_{1,69} = 5.91$, p < 0.05; FLX treatment: $F_{2,69} = 0.139$, NS, and interaction: $F_{2,69} = 0.156$, NS) (Fig. 2, right).

The results obtained in middle aged subjects are shown in Fig. 3. Middle aged females also showed a reduced immobility after 5 and

10 mg/kg of FLX, although of a lesser magnitude than in young adults because the levels of significance decreased from p < 0.001 to p < 0.01. Similarly, in males only the highest dose of 10 mg/kg had an effect, which was of a lower extent than that found in young adults. In middle-aged subjects the results of the two way ANOVA for immobility were: sex, $F_{1,56} = 60.57$, p < 0.001; FLX treatment, $F_{2,56} = 10.92$, p < 0.001 and interaction: $F_{2,56} = 0.49$, NS. FLX increased swimming in both sexes that reached statistical significance only in females, mainly, after the low dose (5 mg/kg). Interestingly, the expression of swimming was always greater in females than males. Climbing trended to increase in both sexes after the highest dose of this SSRI. The two way ANOVAs considering the factors sex and treatment for swimming and climbing were: sex: $F_{1,56} = 85.22$, p < 0.001; FLX treatment: $F_{2,56} = 8.73$, p < 0.001 and interaction: $F_{2,56} = 0.77$, NS and sex: $F_{1,56} = 0.14$, NS; FLX treatment: $F_{2,56} = 4.05$, p < 0.05 and interaction: $F_{2,56} = 0.49$, NS, respectively.

In senescent rats FLX was only tested at the highest dose of 10 mg/kg, which produced a reduction in immobility in females (Fig. 4, left) that
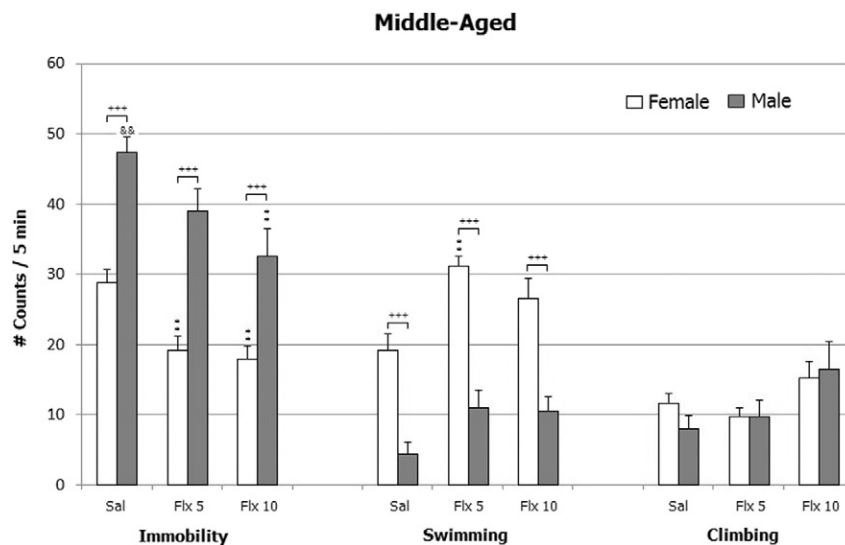
Middle-Aged

Fig. 3. Sex different antidepressant-like effect of fluoxetine (5 and 10 mg/kg) in middle-aged rats (12–15 months old) in the forced swim test. For ANOVA results see text. Asterisks over columns represent differences versus control saline treated animals, **p < 0.01. Sex differences are shown by brackets, $^{+++}$p < 0.001. Age differences are shown by $^{\&\&}$p < 0.01 versus the male senescent saline group. Holm–Sidak post hoc test.
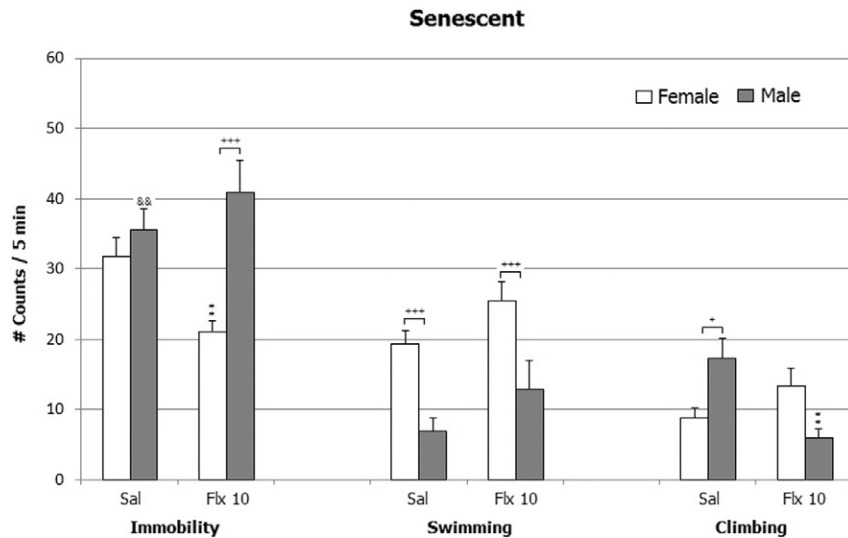
Senescent

Fig. 4. Sex different antidepressant-like effect of fluoxetine (10 mg/kg) in senescent rats (23–25 months old) in the forced swim test. For ANOVA results see text. Asterisks over columns represent differences versus control saline treated animals, $^{**}p < 0.01$. Sex differences are shown by brackets, $^{+}p < 0.05$, $^{+++}p < 0.001$. Age differences are shown by $^{\&\&}p < 0.01$ versus young male adult saline group. Holm–Sidak post hoc test.

was only significant at a $p < 0.05$, suggesting a lower sensitivity to this antidepressant compared to that found in young adults. Remarkably, in senescent males this FLX dose was completely incapable to produce an antidepressant-like effect. Thus the results of the two way ANOVA showed a clear effect of sex ($F_{1,36} = 13.66$, $p < 0.001$), a lack of action of FLX treatment ($F_{1,36} = 0.701$, NS), and a significant interaction between both factors ($F_{1,36} = 6.44$, $p < 0.05$). The reduced immobility in females was at an expense of a slight increase in swimming, although in males, FLX also increased this parameter (two way ANOVAs for sex: $F_{1,36} = 21.83$, $p < 0.001$; FLX treatment, $F_{1,36} = 5.15$, $p < 0.05$ and interaction $F_{1,36} = 0.01$, NS) (Fig. 4, centre). As aforementioned in control animals there was a sex difference in the levels of climbing favoring males, while treatment with FLX reduced this parameter in this sex without modifying it in females (two way ANOVAs for sex: $F_{1,36} = 0.04$, NS; FLX treatment, $F_{1,36} = 1.71$, NS and interaction $F_{1,36} = 9.28$, $p < 0.01$) (Fig. 4, right).

In agreement with previous findings (Olivares-Nazario et al., 2015) old males showed less locomotion than young adults and middle-aged subjects ($F_{2,32} = 12.15$, $p < 0.001$). Aged females, by contrast, show similar levels of locomotion than young adults ($F_{2,42} = 0.526$, NS). As previously shown (Olivares-Nazario et al., 2015; Recamier-Carballo et al., 2012) FLX reduced locomotion in young adults (female: $F_{2,52} = 6.45$, $p < 0.01$ and male: $F_{2,21} = 6.12$, $p < 0.01$) (Table 1). However, FLX treatment particularly at the highest dose (10 mg/kg) and only in old males drastically interfered with motor coordination tested in the rotarod ($F_{2,21} = 12.15$, $p < 0.001$) (Table 2).

4. Discussion

The main findings of the present paper are:

1. Sex and age differences in control animals: (a) young adult and middle-aged females showed lower levels of immobility and higher values of swimming in the FST as compared with males; (b) this sex difference vanishes in senescent animals when males decreased their levels of immobility and increased climbing.
2. Sex and age differences in FLX antidepressant-like effects: (a) Young adult and middle-aged females were more sensitive than males to the antidepressant-like effect of FLX, because they responded to 5 and 10 mg/kg, while males only to 10 mg/kg. (b) Middle-aged animals, regardless of their sex, were less sensitive to the antidepressant-like effect of FLX. (c) FLX at 10 mg/kg produced an antidepressant-like effect in senescent females that was completely absent in males.
3. Senescent males, but not females, showed motor incoordination after FLX (10 mg/kg).

4.1. Aging increases susceptibility to stress

Only in females there was a trend to show more immobility along aging, suggesting that aged rats are more vulnerable to develop despair. In a previous study (Recamier-Carballo et al., 2012) we showed that middle-aged females clearly have higher depressive-like behavior than their young adult counterpart. The main difference between both

Table 2
Effect of fluoxetine (5 and 10 mg/kg) on motor coordination in young adult, middle-aged and senescent female and male rats.

	Young adults		Middle-aged		Senescent	
Treatment	Female	Male	Female	Male	Female	Male
Saline	100/5	100/44	100/30	90/67	100/36 *	57/88
	0	0	0	2	0	3.5 $^{\&\&}$
FLX 5 mg/kg	100/7	100/29	90/33	73/88 *		
	0	0	0	2.5 $^{\# \&}$		
FLX 10 mg/kg	100/30	100/17	89/25	92/45	89/38	63/100 *
	0	0	0	0	0	9 $^{\#\#\# +++ \&\&}$

Table shows percentage of animals performing the test/percentage of animals falling from the rotarod. Numbers under these proportions show the median number of falls. Percentages were compared using the Fisher F test, * $p < 0.05$ versus young adult same sex and treatment. Number of falls was compared by a one way ANOVA followed by Holm–Sidak pos hoc test: $^{\#}$ $p < 0.05$, $^{\#\#\#}$ $p < 0.001$ versus young adult same sex and treatment; $^{+++}$ $p < 0.001$ versus middle-aged same sex and treatment; $^{\&}$ $p < 0.05$, $^{\&\&}$ $p < 0.01$ versus females same age and treatment.

studies rely in the fact that in this study females were not ovariectomized while in the previous one females were tested three weeks after ovariectomy. The finding that ovariectomy increases immobility, particularly in aged animals, is in line with previous results showing that ovariectomized 18 months old rats show more immobility than those tested at six months (de Chaves et al., 2009). In addition, we and others have found high estradiol levels in middle aged diestrus rats as compared with young females in the same estrous-cycle phase (Gore et al., 2000; Olvera-Hernandez et al., 2013; Wise and Ratner, 1980), reinforcing the notion that the rat's ovary keeps secreting estrogen along the whole animal's life (Lu et al., 1979; vom Saal and Finch, 1988). These data, taken together, support the idea that steroid hormones, particularly estradiol, protects against the deleterious effects of stress in aged females (Walf and Frye, 2010). In males we failed to find an increased immobility along aging. Such observation agrees with previous own and others findings (Kasckow et al., 2005; Olivares-Nazario et al., 2015), but contrast with reports showing an increased immobility in aged males as compared with young ones (Frye et al., 2010; Moretti et al., 2011; Onodera et al., 2000). The reasons underlying these differences are unclear but strain and species differences could be involved (Kokras et al., 2015).

In the chronic mild stress (CMS) we have found that 90% and 73% of middle-aged ovariectomized females and males, respectively, showed a diminished sucrose intake as compared with 66% and 35% of young adult ovariectomized females and males that displayed a similar decrease (Recamier-Carballo et al., 2012; Recamier-Carballo and Fernández-Guasti, 2012), indicating that, as in the FST, aging increases the vulnerability to develop anhedonia. It could be argued that such sex difference is also due to gonadal hormones; however, recent findings revealed that orchidectomized young adult males showed a similar resistance to CMS than non-castrated ones and that testosterone restitution to middle aged males prevented anhedonia (Herrera-Perez et al., 2012). Thus, as in females, testicular hormones also seem to play an essential protective role in aged animals (Herrera-Perez et al., 2012; Herrera-Perez et al., 2013; Herrera-Perez et al., 2015). The CMS has the limitation that only young adult (3–5 months) and middle-aged (12–14 months) animals can be compared because 24 months old rats subjected to CMS lost body weight until finally most died (Herrera-Perez et al., 2008). The detailed mechanisms that promote depressive-like behaviors in aged subjects have been reviewed elsewhere (Cameron and McKay, 1999; Hayashi et al., 2001; Herrera-Perez et al., 2015, 2008; Lebowitz et al., 1997; Reynolds and Kupfer, 1999).

4.2. Depressive-like behavior changes according to sex

It has been recently reported that males and females show a different temporal display of immobility and active behaviors in this test (Kokras et al., 2015), for example only in males there is a normal immobility distribution along time. We and others have found that young adult and middle-aged females show less immobility than males (Alonso et al., 1991; Barros and Ferigolo, 1998; Brotto et al., 2000; Contreras et al., 1995; Gomez et al., 2014; Kokras et al., 2009), while others have reported that females show an increased vulnerability to an open space swim test (Sun and Alkon, 2006) and higher immobility than males in the FST (Dalla et al., 2008; Kokras et al., 2015; Pitychoutis et al., 2011; Wibrand et al., 2013) with or without influence of the estrous cycle (Kokras et al., 2015; Kokras et al., 2012). Even a third subset has found no sex differences [for review see (Kokras et al., 2015)]. The reasons underlying these findings could rely on the stress sequence exposure, as has been previously explained (Gomez et al., 2014). Other causes such as handling, strain, methodological approaches and factors influencing FST have been thoroughly detailed in a recent review (Kokras et al., 2015). Interestingly, exposure to the FST produced a sex differential effect in monoamine levels, their metabolites and some of their receptors (Dalla et al., 2008; Drossopoulou et al., 2004). Thus, in females, FST reduced the serotonergic activity in the hypothalamus and the hippocampus, while the dopaminergic activity did not change. Meanwhile in males FST increased both monoaminergic activities in the hippocampus. These differences may underlie the sex behavioral pattern. In the present study non-ovariectomized females were used and, as previously stated, estrogens produce an antidepressant-like effect in this paradigm [for review see: (Estrada-Camarena et al., 2010)] suggesting that ovarian hormones may mediate the reduced immobility found in this sex. However, against this idea we have recently reported (Gomez et al., 2014) that the lower levels of immobility in females, when compared to males, remains unaltered after adult ovariectomy, suggesting that it does not rely on the activational effects of steroids in the adult animal. Interestingly, in the very old subjects the sex difference in basal levels of immobility vanishes, old males show less immobility and higher climbing. These finding are similar to those found by others (Hill et al., 2003) who reported that 18 months old male rats had lower levels of immobility than females, possibly due to lower levels of corticosterone.

4.3. Antidepressant-like response to fluoxetine varies according to sex

The present results revealed that young adult females, regardless of the estrous cycle phase, were more sensitive than young adult males to the antidepressant-like effects of FLX. This result is in agreement with previous own data using FLX (Gomez et al., 2014) and with other SSRIs, like paroxetine (David et al., 2001b) or sertraline (Kokras et al., 2015) or with a tricyclic with primarily serotonergic activity such as clomipramine (Marvan et al., 1997). In line, females showed an increased sensitivity to the anxiolytic-like effects of 5-HT$_{1A}$ agonists (Blanchard et al., 1992). Interestingly, the sex difference in FLX antidepressant-like action does not seem to be influenced by ovarian steroids because (a) adult ovariectomy does not alter the sensitivity to FLX (Gomez et al., 2014), and (b) the antidepressant-like effect of FLX did not change depending upon the estrous cycle phase (present results). The sex-differences in the antidepressant-like effect of FLX and other compounds with antidepressant-like effects in young adult subjects have been previously discussed in detail (Dalla et al., 2010; Estrada-Camarena et al., 2011a; Frye, 2011; Gomez et al., 2014; Kokras et al., 2015).

A similar sex difference to that found in young adults was observed in middle-aged rats: females show a clear antidepressant-like action of FLX at 5 and 10 mg/kg, while males only responded to 10 mg/kg. Such finding agrees with previous results reporting that young (4 weeks old) and older (3,6, and 14 months old) animals are similarly sensitive to the antidepressant-like effect of imipramine in the novel open-space swim test (Sun and Alkon, 2008). By contrast, in the present study we found that in very old animals, FLX reduced immobility in females while it completely lacked an effect in males. Unfortunately the literature of sex differences in antidepressants' actions is scarce in the aged population. Previous data showed a drastic sex differential anticompulsive effect of FLX (10 mg/kg) in middle-aged rats in an animal model of obsessive compulsive disorder (OCD) (Olvera-Hernandez et al., 2013): fluoxetine had an action in females and not in males. Such sex difference most likely resides in the dissimilar serotonin system function between sexes (Carlsson and Carlsson, 1988; Jovanovic et al., 2008). These data, taken together, reinforce the idea that females respond better to drugs acting at the serotonergic system and suggest that along aging the sex difference is more noticeable because males cease responding. In consequence the main difference in FLX action along aging involves males.

In agreement with present findings a reduced or absent effect of antidepressants in aged male rodents has been indicated (David et al., 2001a; Herrera-Perez et al., 2010; Hill et al., 2003; Olivares-Nazario et al., 2015). The lack of antidepressant-like effect of FLX in very old males does not seem to be a dose-related problem because 20 mg/kg also failed to reduce immobility (Olivares-Nazario et al., 2015). Interestingly, some have reported that aged females (12–18 months) keep

responding to classic antidepressants (Recamier-Carballo et al., 2012; Romano-Torres and Fernández-Guasti, 2010), to the antidepressant-like effects of melatonin (Hill et al., 2003) or estrogens (Recamier-Carballo et al., 2012; Romano-Torres and Fernández-Guasti, 2010; Walf and Frye, 2010), while others (Kiss et al., 2012) have claimed a lack of response to this steroid. This controversy might be underlied by the ovariectomy time that does not seem to influence FLX's antidepressant action (Estrada-Camarena et al., 2011b). The main pharmacological target of FLX is the serotonin pathway that is deteriorated in aged subjects (Duncan and Hensler, 2002; Herrera-Perez et al., 2013; Hussain and Mitra, 2000; Kakiuchi et al., 2001; Lawlor et al., 1989; van Dyck et al., 2000; Yamamoto et al., 2002; Yau et al., 1999). This deterioration may be a potential mechanism to explain the reduced efficacy of FLX in males. It remains to be established whether there is a sex differential decline in the serotonergic system that would clarify the sex differences here reported.

4.4. Side effects of fluoxetine do not interfere with its antidepressant action

The careful analysis of motor parameters showed that the antidepressant-like effect of FLX was not a consequence of unspecific motor activation, excluding the possibility of false-positive interpretations. Indeed, in some groups locomotion was decreased by FLX, as reported for most antidepressants (Cryan et al., 2005), whereas it was not increased by any dose. Notably, FLX may produce an antidepressant-like effect despite some motor actions, while the lack of an antidepressant-like effect of FLX in old males did not seem the consequence of a reduced ambulation.

Remarkably, clear sex differences in FLX's motor incoordination were found: in females there was no effect, while in males, the high dose (10 mg/kg) in the oldest population (23–25 months) increased the number of falls and reduced the proportion of subjects able to perform the test. This observation is in line with previous results (Olivares-Nazario et al., 2015; Olvera-Hernandez et al., 2013) where we found that in middle-aged and old males fluoxetine completely lacked a therapeutic-like action but provoked motor incoordination (measured as a decrease in the proportion of subjects able to perform the task and in the number of falls). These data suggest differential effects of aging on drug targets. Thus, in addition to age-related pharmacokinetic changes that cannot explain the males insensitivity to SSRIs therapeutic-like effects [see (Olivares-Nazario et al., 2015) for discussion], it may be hypothesized that the drugs lack antidepressant- or anticompulsive-like effects in aged males due to a decreased serotonin transporter density in the brain regions controlling these actions; while these same drugs produce excessive side effects due to specific targets over-expression in other brain structures, as has been suggested for benzodiazepines (Wikinski et al., 2001). It remains to be studied whether these targets changes in males, due to aging, are associated to the decrease in gonadal hormone levels and if they occur also in females.

4.5. Implications of sex and age differences in the response to SSRSs

The present better response of females versus males in the antidepressant-like effect of FLX is in line with clinical observations showing gender differences in SSRIs action favoring women (Berlanga and Flores-Ramos, 2006; Kornstein et al., 2000; Martenyi et al., 2001; Thase et al., 1996). Furthermore, this difference does not seem to be limited to their antidepressant action, because Mundo's group (Mundo et al., 1999, 2002) reported that young women suffering from OCD respond better than men to the anticompulsive effect of clomipramine, possibly due to sex-differences in metabolic characteristics.

The data here shown also indicate a lower response of aged males to the antidepressant-like effect of FLX, an observation in line with the reduced antidepressant effectiveness in older patients (Lenze et al., 2008; Sheffrin et al., 2009; Tedeschini et al., 2011). Interestingly,

a meta-analysis of placebo-controlled randomized trials (Tedeschini et al., 2011) showed that a large variety of antidepressants were more effective than placebo in young adult patients than in their counterpart aged 55 years or older. Indeed, in the elderly subset (>65 years) the antidepressant and placebo groups were alike, but there was significant heterogeneity in the results, possibly due to gender differences. The careful analysis of the effectiveness of antidepressants in very old patients may suggest that a better response is obtained in women cohorts. Thus, for example, some authors (Trappler and Cohen, 1998) have reported that in very old depressed patients, with a mean age of 89 years, the three SSRIs, fluoxetine, sertraline or paroxetine, produced clear antidepressant effects with no differences between them and with good tolerance. Remarkably, this population consisted in 79% women.

In clinics clarifying the relevance of sex, age and drug interaction is difficult because most reports examining the effects of antidepressants do not discuss this issue. It is unclear if the shortage of reports reflects a failure to test sex differences or underreporting of negative results. Finally, although gender differences in treatment response are not large enough in young adults, they may intensify in very old patients who are more susceptible to develop this illness. These findings suggest that gender should guide the clinical use of specific antidepressants particularly in the elderly and raise the possibility that they work differently in men and women of different ages.

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