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Acute administration of fluoxetine increases social avoidance and risk assessment behaviors in a sex- and social stress-dependent manner in Syrian hamsters (*Mesocricetus auratus*)

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Abstract

Most studies investigating the effects of acute administration of selective serotonin reuptake inhibitors (SSRI) on responses to social stress have been conducted with males. This is despite the fact that SSRIs remain the primary pharmacotherapy for social stress-related disorders for both sexes and that the prevalence of these disorders is twofold higher in women than in men. To determine whether acute treatment with the SSRI, fluoxetine, alters behavioral responses to social defeat stress in a sex- or social stress-dependent manner, male and female Syrian hamsters were subjected to one of three social defeat conditions: no defeat (placed into an empty resident aggressor (RA) cage), a single defeat by one RA for 15 minutes, or three consecutive defeats using different RAs for five minutes each. The day following social defeat, subjects were infused with either vehicle or fluoxetine (20 mg/kg, I.P.) two hours prior to a five minute social avoidance test. Overall, we found that fluoxetine increased social vigilance regardless of sex or defeat condition. We also found that fluoxetine affected social avoidance in a sex by stress intensity interaction, such that fluoxetine increased avoidance in no defeat males and in males defeated once but significantly increased avoidance in females only after three defeats. These data suggest that treatment with an SSRI could initially exacerbate the effects of social stress in both sexes. These data also emphasize the importance of including sex as a biological variable when investigating the efficacy of pharmacotherapy for stress-related disorders.

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Conflicts of interest:
The authors have no conflicts of interest to declare.

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Keywords
Social defeat; Social stress; SSRI; fluoxetine; vigilance; anxiety; sex differences

1. Introduction

Social stress is the most common stressor experienced by humans and is positively associated with many trauma- and stress-related neuropsychiatric disorders, such as major depressive disorder (MDD) and post-traumatic stress disorder (PTSD) (Heim and Nemeroff, 2001; Huhman, 2006). Social defeat models have been widely used to model social stress in humans and to improve our understanding of the pathophysiology of these trauma- and stress-related neuropsychiatric disorders (Golden et al., 2011; Huhman, 2006; Huhman et al., 2003). Beyond a mechanistic understanding, though, these social defeat models are also useful for investigating the efficacy of frontline pharmacotherapies for those experiencing social stress and those suffering with social stress-related neuropsychiatric disorders.

The most prescribed class of pharmacotherapies for trauma- and stress-related neuropsychiatric disorders, including MDD and PTSD, are the selective serotonin reuptake inhibitors (SSRIs) (Friedman and Sonis, 2020; Hayes et al., 2012). While the efficacy of chronic SSRI treatment to lower anxiety (Berton et al., 1999; Berton et al., 2006; Krishnan et al., 2007; Sial et al., 2016) and to decrease social avoidance (Berton et al., 2006; Cao et al., 2010; Dekeyne et al., 2000; Razzoli et al., 2011; Sial et al., 2016; Van Ameringen et al., 1993) has been widely reported in laboratory rodents and humans, it may be that acute treatment with SSRIs enhances anxiety (Drapier et al., 2007; Kurt et al., 2000; Robert et al., 2011; Silva and Brandao, 2000) and increases social avoidance (Payet et al., 2018; Payet et al., 2021), counter to the desired effects of treatment. This is clinically relevant when treating patients with MDD and PTSD, where already high levels of social withdrawal (Hofmann et al., 2003; Ullman and Filipas, 2005) might be exacerbated by initial SSRI treatment.

Importantly, almost all basic studies investigating the efficacy of SSRIs in modulating social anxiety and avoidance have been conducted with males (Berton et al., 2006; Cao et al., 2010; Dekeyne et al., 2000; Drapier et al., 2007; Payet et al., 2018; Razzoli et al., 2011; Sial et al., 2016; Van Ameringen et al., 1993). This is, in part, because of challenges associated with defeating female mice and rats (Haller et al., 1999; Harris et al., 2018; Newman et al., 2019; Takahashi et al., 2017). However, both male and female Syrian hamsters readily engage in territorial aggression and experience social defeat (Albers et al., 2002; Bastida et al., 2009; Gattermann et al., 2001; Lai et al., 2005; Lai and Johnston, 2002), allowing for ethologically relevant examinations of behavioral responses to social stress in both sexes (Huhman et al., 2003). Examining the relationship between acute SSRI treatment and social stress in females is essential, given that females are more than twice as likely to be diagnosed with either MDD or PTSD than are males (Haskell et al., 2010; Kessler, 1997, 2003; Kessler et al., 1993; Kessler et al., 1995), that there are basal sex differences in susceptibility to social stress in animals models (Bath and Johnston, 2007; Huhman et al.,...
2003; Trainor et al., 2011), and that there is already evidence that SSRIs can alter social behaviors in a sex-dependent manner (Greenberg et al., 2014; Terranova et al., 2016).

It is also important to note that most studies to date have only used a single intensity of social defeat stress (e.g., 10 days of social defeat) to examine the effects of fluoxetine on social avoidance and risk assessment behaviors (Berton et al., 1999; Berton et al., 2006; Cao et al., 2010; Razzoli et al., 2011), which prevents examining the relationship between SSRI treatment and the intensity of social defeat on social avoidance and risk assessment behaviors. There is evidence that males and females have different “dose response curves” to social stimuli (Borland et al., 2019a; Borland et al., 2018; Borland et al., 2019b; Feng et al., 2015). Therefore, different intensities of social stress might interact with fluoxetine to increase social avoidance and risk assessment behaviors sooner (i.e., at a lower "dose") in one sex than the other.

Here, we test the hypothesis that acute treatment with the SSRI, fluoxetine, increases social avoidance and risk assessment behaviors in a sex- by social stress-dependent manner. First, we examined the effects of social stress intensity on social avoidance and risk assessment behaviors and found that both males and females have a linear increase in both social avoidance and social vigilance as intensity, or “dose”, of the stressor increases from a novel cage exposure to a single defeat to three defeats. We then tested whether fluoxetine would alter this “dose-response” relationship in a sex-dependent manner.

2. Methods

2.1. Subjects

Adult male and female Syrian hamsters (n = 111 males and 109 females) were obtained from Charles River Laboratories (Wilmington, MA) at approximately 2 months of age and weighing between 120g and 130g. Animals were run at different times in three, separate cohorts. Each time upon arrival, animals were individually housed for 2 to 3 weeks before behavior testing in 24cm × 33cm × 20cm polycarbonate cages filled with corncob bedding and nesting material. It is important to note that individual housing is not stressful for Syrian hamsters (Ross et al., 2017). Food and water were available ad libitum. Prior to behavioral testing, females’ cycles were monitored for eight days to confirm regular cyclicity, with males handled similarly each day. In addition to our male and female test subjects, male and female resident aggressors (RAs) were also used. RAs were larger, individually-housed Syrian hamsters, and in the case of female RAs, were ovariectomized to avoid estrous cycle variation in aggressive behavior (Solomon et al., 2007). All procedures and protocols were performed in accordance with the principles of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at Georgia State University.

2.2. Social defeat training

To test the effects of acute fluoxetine administration on social stress responses, we employed a social defeat procedure used previously (Huhman et al., 2003; McCann and Huhman, 2012; Potegal et al., 1993). Briefly, on a day of Diestrus, females and yoked males were
taken to the behavioral testing suite. Once there, the subjects in the no social stress group (i.e., no defeat controls) were placed into the empty cage of a same-sex RA for one, 15 min session or three, 5 min sessions in a novel, empty RA cage as described in more detail below. This group controlled for the effects of all variables except social defeat. For the “medium” social stress group (i.e., 1×15 min defeat), we placed subjects into the cage of a same-sex RA for 15 min. After 15 min, subjects were returned to their home cages. For the “high” social stress group (i.e., 3×5 min defeats), we placed subjects into the cage of a same-sex RA for 5 min for three defeat trials separated by 5 min intertrial intervals wherein subjects were placed back in their home cages. A novel RA was used for each of the three defeat trials. We know from long experience with this model that, despite the fact that the medium and high stress groups are exposed to the same overall minutes of social stress, animals exposed to three different RAs will respond with more social avoidance than will animals exposed to only one RA (unpublished data).

2.3. Drug administration and social avoidance testing

The day following defeat, the no defeat controls and defeated subjects were injected intraperitoneally with either fluoxetine (20 mg/kg) or saline vehicle approximately 2 hours before testing. We have previously demonstrated that this dose of fluoxetine alters attack latency in male and female Syrian hamsters in a sex-dependent manner (Terranova et al., 2016). Two hours following injection, subjects were placed into a novel, clean cage facing away from an unfamiliar RA that was confined in a plastic mesh cage (13.5cm × 13.5cm × 7cm) that allowed the test subjects to smell, hear, and see the RA without allowing any direct contact between the animals for 5 min. We measured social avoidance, social vigilance, and social investigation in the subjects. Social avoidance was operationally defined as the time subjects spent on the half of the testing cage opposite the caged RA. Social vigilance was operationally defined as subjects standing on their back legs with head oriented towards the caged RA or subjects being in a frozen stretch attend position with their head oriented towards the caged RA. Finally, social investigation was operationally defined as the time that subjects spent with their snouts touching the mesh cage containing the RA.

2.4. Statistics

Data were analyzed using SPSS version 28 (IBM). A 2×2×3 between-subjects analysis of variance (ANOVA) was run with sex, drug treatment, and testing conditions as the factors. In cases of statistical significance, Fisher’s Least Significant Difference (LSD) post-hoc tests were used to examine group differences. Main effects were reported if they were informative above and beyond interaction effects. Given that there were no statistical differences between our no defeat control conditions, these groups were collapsed for final analyses. Partial η² (η²P) are reported as measures of effect sizes from the ANOVAs. All data are presented as mean ± standard error of the mean. Statistical significance was conferred at p < 0.05.
3. Results

3.1. Effects of sex, drug treatment, and defeat conditions on social avoidance

The same pattern of results was observed within each of the three cohorts of the experiment, and there were no significant differences among the cohorts (p > 0.05), therefore all data were pooled and analyzed together. There was a significant effect of defeat condition on social avoidance (F(2, 208) = 70.82, p < 0.01, η²_p = 0.39; Figure 1), such that there was a stepwise increase in social stress across condition (i.e., no defeat controls exhibited the least social avoidance, subjects defeated 1×15 min had an intermediary amount of social avoidance, and subjects defeated 3×5 min produced the most social avoidance; all were significantly different from each other). There was also an interaction between sex, drug treatment, and defeat condition F(2, 208) = 6.51, p < 0.01, η²_p = 0.06; Figure 1), such that fluoxetine increased social avoidance in males but not females in the no defeat (Males: p < 0.01; Females: p = 0.09) and 1×15 min defeat (Males: p < 0.01; Females: p = 0.24) groups, and increased social avoidance in females but not males that were defeated 3×5 min (Males: p = 0.24; Females: p < 0.01).

3.2. Effects of sex, drug treatment, and defeat conditions on social vigilance

There was a significant effect of defeat condition on social vigilance (F(2, 208) = 23.10, p < 0.01, η²_p = 0.18; Figure 2), such that social vigilance increased after social defeat. There was also a main effect of drug treatment, such that fluoxetine-treated subjects exhibited higher social vigilance than did saline-treated controls (F(1, 208) = 68.57, p < 0.01, η²_p = 0.25; Figure 2). There were no significant interactions between sex, drug treatment, or defeat conditions on social vigilance (p’s > 0.05).

3.3. Effects of sex, drug treatment, and defeat conditions on social investigation

There was a significant effect of defeat condition, such that females investigated more than males (F(1, 208) = 9.42, p < 0.01, η²_p = 0.04; Figure 3). There was also an interaction between sex, drug treatment, and defeat condition (F(2, 208) = 6.51, p < 0.01, η²_p = 0.06; Figure 3), such that fluoxetine decreased social investigation in males but not females defeated 1×15 min (Males: p = 0.01; Females: p = 0.90) and decreased social investigation in females but not males defeated 3×5 min (Males: p = 0.54; Females: p < 0.01).

4. Discussion

Here, we tested the hypothesis that acute treatment with the SSRI, fluoxetine, would increase social avoidance and risk assessment behaviors in a sex- by social stress-dependent manner. In partial support of our hypothesis, we found that acute fluoxetine increased social avoidance and decreased social investigation in males, but not females, after no social stress or an intermediate social stresor, whereas acute fluoxetine increased social avoidance and decreased social investigation in females only after exposure to a stronger stresor (Fig. 1). This could be related to underlying sex differences in serotonin receptor densities in Syrian hamsters (Grieb et al., 2021; Ross et al., 2019). For example, male Syrian hamsters have more serotonin 1A receptors in the bed nucleus of the stria terminalis than do females, a region where serotonin acts to increase anxiety (Hammack et al., 2009; Marcinkiewcz...
et al., 2016). Therefore, males may have higher basal levels of social avoidance and risk assessment behaviors and be more sensitive to the anxiogenic effects of acutely administered serotonin-targeting drugs than are females. Consistent with this idea is our finding that no defeat males were more socially avoidant than were no defeat females, that acute fluoxetine increased social avoidance in males at lower levels of stress than females, and that males reached their ceiling for social avoidance after fluoxetine treatment earlier on the social defeat “dose-response curve” than did females (Fig. 1).

It is also interesting to note that acute fluoxetine increased social vigilance regardless of sex or social defeat (Fig. 2). This is despite the fact that sex interacted with social defeat condition to affect social avoidance. This could suggest that acute fluoxetine might generally increase social anxiety-like behaviors but that a lower stressor threshold is needed to affect social vigilance than is necessary to affect social avoidance. This is particularly relevant when considering that no defeat females display more social approach than do males (Fig. 3; Borland et al., 2019a; Borland et al., 2018; Borland et al., 2019b). This could suggest that while acute fluoxetine increases social vigilance in males and females regardless of social stress intensity, it may take high social stress (i.e., 3 defeats) before it is able to overcome females’ naturally higher tendency towards social approach.

The present data suggest that acute fluoxetine treatment increases social vigilance regardless of sex or social stress condition. This finding suggests that initial treatment with SSRIs during pharmacotherapy may initially increase social vigilance and exacerbate social anxiety. This could be particularly important when considering SSRIs for the treatment of trauma- and stress-related neuropsychiatric disorders, as these disorders are already associated with higher vigilance to negatively-valanced social stimuli (Dalgleish et al., 2001; Vassilopoulos, 2005). In contrast to social vigilance, acute fluoxetine treatment increased social avoidance in a sex- and stress-dependent manner. This could be crucial when treating patients with trauma- and stress-related neuropsychiatric disorders, who already have high levels of social withdrawal or avoidance (Hofmann et al., 2003; Ullman and Filipas, 2005). Taken together, these data suggest that initial treatment with SSRIs during pharmacotherapy could exacerbate depression- and anxiety-related symptoms in men and women. It should be noted, however, that pharmacotherapy in humans occurs sometimes months after stressor exposure, rather than immediately after as in this study, which may have further exacerbated the effects of acute SSRI treatment on social avoidance and risk assessment behaviors.

Furthermore, other studies have found that SSRI dose can interact with sex to drive social avoidance (Greenberg et al., 2014). Therefore, a lower dose of fluoxetine might be more or less effective at disrupting social avoidance compared to the dose used here. These data also suggest that females may be more resilient to detrimental effects of acute fluoxetine on social anxiety than are males, particularly when exposed to less intense social stress. Finally, these data emphasize the importance of including sex as a biological variable and of including varying intensities of social stress when investigating the efficacy of pharmacotherapies for social stress-related neuropsychiatric disorders.

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### Highlights

- Acute fluoxetine increases social vigilance regardless of sex or defeat condition
- Acute fluoxetine affected social avoidance in a sex by stress intensity interaction
- Acute fluoxetine increased social avoidance in males after one defeat
- Acute fluoxetine increased social avoidance in females after three defeats
Figure 1.
Duration of social avoidance operationally defined as time on the far side of the cage by saline-treated or fluoxetine-treated, male and female Syrian hamsters who were not socially defeated (no defeat), were defeated one time for 15 minutes (1×15) or were defeated three times for five minutes each (3×5). Number inside bars indicate the number of subjects per group. All values are expressed as Mean ± SEM. * indicates significant differences between saline and fluoxetine treatment, $p < 0.05$. 
Figure 2.
Duration of social vigilance operationally defined as time standing on their back legs with head oriented towards the caged RA or time being in a frozen stretch attend position with their head oriented towards the caged RA by saline-treated or fluoxetine-treated, male and female Syrian hamsters who were not socially defeated (no defeat), were defeated one time for 15 minutes (1×15) or were defeated three times for five minutes each (3×5). Number inside bars indicate the number of subjects per group. All values are expressed as Mean ± SEM. * indicates main effects, p < 0.05.
Figure 3.
Duration of social investigation operationally defined as time the subjects’ nose contacted the mesh cage housing a confined resident aggressor by saline-treated and fluoxetine-treated, male and female Syrian hamsters who were not socially defeated (no defeat), were defeated one time for 15 minutes (1×15) or were defeated three times for five minutes each (3×5). Number inside bars indicate the number of subjects per group. All values expressed as Mean ± SEM. * indicates differences between saline and fluoxetine treatment, p < 0.05.