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Attenuation of compulsive-like behavior by fluvoxamine in a non-induced mouse model of obsessive-compulsive disorder

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Abstract

The current study evaluated the role of strain and compulsive trait differences in response to fluvoxamine, a common OCD drug, in two different mouse strains (BIG1 and BIG2) exhibiting a spontaneous compulsive-like phenotype. For compulsive-like nest-building behavior, dose-dependent attenuation of nesting by fluvoxamine was observed for the BIG1 compulsive-like strain during the first hour after administration. No significant differences were found for BIG2 strain during the first hour, although a dose-dependent trend similar to that seen in the BIG1 strain was observed. Fluvoxamine dose-dependently decreased the number of marbles buried in both strains one hour after administration. For anxiety-like behaviors in the open field, no significant drug effects were found for the latency to leave the center and the number of line crossings. Significant strain differences were observed, with the BIG2 strain exhibiting higher anxiety-like behaviors and reduced locomotor activity when compared to the BIG1 strain. Consequently, this study adds predictive validity to our mouse model of OCD, while the anxiety-like differences between the strains add heterogeneity to our mouse model, similar to the heterogeneity seen in OCD.

Keywords

Fluvoxamine; obsessive-compulsive disorder; mouse model; strain; trait; anxiety

Introduction

Obsessive-compulsive disorder (OCD) is an intricate heterogeneous disorder characterized by persistent obsessions (thoughts) and compulsions (repetitive behaviors) (Albert et al., 2013; Karno et al., 1988). In the United States OCD has an estimated lifetime occurrence of about 2.3% (Ruscio et al., 2010). The social functioning, relationships, quality of life and socio-economic status of patients suffering from OCD are significantly impacted (Fontenelle et al., 2010; Hollander et al., 2010). The most common first-line treatments for OCD are selective serotonin reuptake inhibitors (SSRIs) and cognitive behavioral therapy (Bandelow

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et al., 2012; Kellner, 2010; Pittenger et al., 2005). Fluvoxamine is a first generation SSRI widely used for treatment of OCD (Irons, 2005). It is a potent inhibitor of serotonin reuptake at synapses and has no effect on reuptake of dopamine or norepinephrine (Goodman et al., 1997). Many clinical studies have shown efficacy of fluvoxamine in treating OCD (Goodman et al., 1989a, Mallya et al., 1992; Milanfranchi et al., 1997; Mundo et al., 2001). However, 40–60% of the OCD patients are refractory to the first-line treatments (Nakamae, 2013). Even patients categorized as clinical responders continue to exhibit impairments from their residual obsessions and compulsions (Lack, 2012). Considering such high rates of drug resistance among patients, a better understanding of the efficacy of first-line treatments on specific compulsive traits in OCD subjects of different genetic backgrounds is needed.

OCD has a strong genetic component, which has been established in both human and rodent studies (Hettema et al., 2001; Pato et al., 2002; Taylor, 2013; Ting and Feng, 2011; Wang et al., 2009; Welch et al., 2007). Investigating drug intervention in mouse strains exhibiting spontaneous compulsive-like behaviors can be a valuable starting point for determining if there is any role of genetic background and compulsive traits in influencing drug response. The current mouse model was developed by bidirectionally selecting house mice, *Mus musculus*, for nest-building behavior (Bult and Lynch, 2000; Lynch, 1980). The stock population for the original selection experiment (Lynch, 1980) was a cross among eight inbred strains yielding the HS/Ibg outbred strain (McClearn et al., 1970; Lynch, 1980). Nest-building behavior, which is homologous to hoarding in humans with OCD (Warneke, 1993), is considered to be a measure of compulsive-like behavior in mice (Greene-Schloesser et al., 2011). Bidirectional selection resulted in three main levels of nest-building behavior (with two replicate strains within each level). The replicates within each level of nesting were maintained separately and subjected to the same selection regime. Hence, all replicates were maintained as separate strains (Bult and Lynch, 2000; Lynch, 1980). Considering replicate strains for comparison is important to guarantee that responses to artificial selection are the result of selection and not due to founder effects or random genetic drift when comparing the different levels of selection, i.e., selection for building big nests or small nests, or random breeding (Bult and Lynch, 2000; Lynch, 1980). The BIG strains (BIG1 and BIG2) consistently display a forty-fold difference in the amount of cotton used when compared to the SMALL strains which display very low levels of nesting. The randomly-bred Control strains serve as a selection control and show intermediate levels of nesting (Bult and Lynch, 2000; Lynch, 1980). We have shown in the past that the replicates within each level of nesting, although subjected to the same selection regime, diverged genetically, probably due to founder effects or random genetic drift (Bult and Lynch, 1996, 2000).

According to Maio et al. (2014), comparing our compulsive-like strains can be useful for understanding the disease mechanism during the OCD condition. Prior studies have shown that our compulsive-like BIG1 and BIG2 strains exhibited varied levels of compulsive-like and anxiety-like behaviors (Mitra et al., 2016a, 2017). The BIG1 and BIG2 strains therefore exhibit face (Mitra et al., 2017) and construct validity (Mitra et al., 2016a) for subgroups of compulsive-like expression as seen in subsets of human patients. In addition, a previous study with inbred mouse strains also found significant variations in expression of drug-induced compulsive-like behaviors (de Hass et al., 2012). The study showed how the A/J mouse strain had higher susceptibility to quinpirole-induced compulsive-like checking

behaviors when compared to the C57BL6/J mouse strain (de Hass et al., 2012). Though the predictive validity of the BIG1 strains has been previously established (Greene-Schloesser et al., 2011), this is the first time we have investigated the predictive validity of the BIG2 strains and compared them with the BIG1 strains for better understanding of strain- and trait-based influences on drug effectiveness and behavioral expression. Due to the variations seen in behavioral expression between the compulsive-like BIG1 and BIG2 strains (Mitra et al., 2016a, 2017), we hypothesized that there would be a strain- and trait-specific attenuation of compulsive-like and anxiety-like behaviors due to fluvoxamine administration.

Methods

Subjects

Intact compulsive-like BIG male house mouse (*Mus musculus*) strains (BIG1, BIG2) were used for the study (BIG1 n=36; BIG2 n=38). A total of 12 animals per group per strain were used initially, but 12 mice from BIG1 and 10 mice from BIG2 strain were euthanized due to skin scabbing. Mice were raised in polypropylene cages (27×17×12 cm) with wood shavings, under a 12-12 light-dark cycle at 22±1°C, and with free access to food (Purina Mills, Lab Diet Mouse Diet #5015, St. Louis, MO) and water. Pups were weaned at 19–21 days of age and housed with same-sex littermates. All animals were approximately 60 days old at the start of the experiment.

Drug administration

Fluvoxamine maleate (Sigma Aldrich) was dissolved in physiological saline (pH=7.4) to yield final doses of 10 mg/kg, 20 mg/kg and 40 mg/kg (pH 7–7.4). Saline served as a vehicle (0 mg/kg) control. All male compulsive-like mice were divided into four treatment groups (starting at 12 animals per group) comprising vehicle (sterile saline with pH adjusted to 7.4), 10 mg/kg, 20 mg/kg and 40 mg/kg fluvoxamine. Animals in each group received subcutaneous injections of fluvoxamine/vehicle daily for 17 days.

Experimental design

All animals underwent behavioral testing consecutively, comprising nest building on day 15, marble burying on day 16 and open field test on day 17 of the 17-day fluvoxamine treatment regimen. The sequence of behavioral tests, i.e., doing the anxiety-like tests before the compulsive-like tests or vice versa, does not affect the performance in any of these behaviors in our mouse strains (data not shown).

For nest-building, data were collected during the first, second, third, fourth, fifth hour and 24 h after fluvoxamine administration. Marble burying and open field tests were performed one hour after drug administration. The marble burying test lasted for 15 minutes and open field test for 3 minutes (Greene-Schloesser et al., 2011; Mitra et al., 2016a,b, 2017). Testing was performed in the light phase of the light-dark cycle and the University of Alaska Fairbanks Institutional Animal Care and Use Committee approved the animal care and experimental procedures (IACUC assurance number 718349).

Compulsive-like nest-building behavior

Nest-building behavior was used as a measure of the compulsive-like phenotype of the mice (Greene-Schloesser et al., 2011). Male mice of both the BIG strains (BIG1 and BIG2) were singly housed and provided with a pre-weighed cotton roll in the cage-top food hopper. The cotton roll was removed and weighed (Bult and Lynch, 1996, 1997, 2000) 1, 2, 3, 4, 5 and 24 h after fluvoxamine administration. The nesting score was defined as the amount of cotton used over a 1-h period (first, second, third, fourth and fifth hour) and over the 24-hour period.

Compulsive-like marble burying behavior

The marble-burying test was also used to determine compulsive-like behavior (Angoa-Perez et al., 2013; Greene-Schloesser et al., 2011; Takeuchi et al., 2002; Thomas et al., 2009). One hour after fluvoxamine administration, mice were individually introduced into a polypropylene cage (37×21×14 cm) containing 20 glass marbles (10 mm in diameter) spaced evenly on wood chip bedding 5 cm deep, without access to food or water, for 15 min (Greene-Schloesser et al., 2011). Testing was carried out in a testing room separate from the housing room. The total number of marbles buried at least 2/3 in the 15-min period was quantified as compulsive-like digging behavior. After the 15-min test, the animals were returned to their home cages.

Anxiety-like open field behavior

Anxiety-like behavior was evaluated using the open field test (Prut and Belzung, 2003; Simon et al., 1994). BIG1 and BIG2 males were transported in their home cages, singly housed, and placed on a rack outside the testing room just prior to the start of testing. The open field apparatus consisted of an open field arena (40 × 40 × 30 cm) with 16 10×10 cm squares on its floor. For testing, animals were placed in the center of the field and allowed to explore the arena for 3 minutes. Latency to leave the central square (20×20 cm) initially and entries into the central zone (20 × 20 cm) after leaving it initially were recorded to quantify anxiety-like behaviors. The total number of line crossings was recorded as a measure of locomotor activity (Frye et al., 2006; Greene-Schloesser et al., 2011; Nosek et al., 2008). The apparatus was cleaned before each test.

Statistical analysis

Values from all behavioral assessments were expressed in mean ± standard error of the mean (SEM). *Statistical Analysis System Software (SAS Version 9.4, Cary, NC)* was used for statistical analyses. All behavioral measures were tested in a general linear model (GLM) analysis of variance (ANOVA) for effects of strain, drug and strain x drug interaction. If significant effects were found, appropriate post-hoc pair-wise comparisons were conducted using the Tukey's Studentized Range test. The total nesting score was square-root transformed to obtain a more normal distribution (Bult and Lynch, 2000); however, the data are presented as non-transformed nesting scores.

Results

Strain-dependent attenuation of compulsive-like nesting behavior by fluvoxamine

After 1 hour of fluvoxamine administration there was a significant drug effect ($F_{3,74} = 11.73$, $p < 0.001$). The 20 and 40 mg/kg doses significantly attenuated the nesting scores for the BIG1 strain when compared to vehicle (Fig. 1). No significant effect of fluvoxamine on nesting in the compulsive-like BIG2 strain was found, although nest-building behavior tended to decrease with increased doses of fluvoxamine (Fig. 1). The strain effect ($F_{1,74} = 1.40$, NS) and drug by strain interaction ($F_{3,74} = 2.43$, NS) were not significant, which confirmed the trend in the BIG2 strain.

No significant drug effect was found in the second ($F_{3,74} = 1.92$, NS), third ($F_{3,74} = 1.45$, NS), fourth ($F_{3,74} = 1.93$, NS), or fifth ($F_{3,74} = 0.24$, NS) hour, or over the whole 24 hours after fluvoxamine administration ($F_{3,74} = 2.01$, NS) (data not shown).

Dose-dependent attenuation of compulsive-like marble burying behavior by fluvoxamine in both the BIG strains

A significant dose-dependent drug effect ($F_{3,74} = 13.08$, $p < 0.001$) was observed in the number of marbles buried. The 20 and 40 mg/kg doses showed significant attenuation of marble burying when compared to vehicle in both the BIG1 and BIG2 strains. No significant strain effect ($F_{1,74} = 1.99$, NS) and drug by strain interaction ($F_{3,74} = 0.88$, NS) were found (Fig. 2).

Significant strain differences in anxiety-like open field behavior

For anxiety-like behavior in the open field, there was a significant strain effect ($F_{1,74} = 39.83$, $p < 0.001$) for the latency to leave the central square, with the BIG1 strain taking less time when compared to the BIG2 strain. The drug effect ($F_{3,74} = 2.21$, NS) and drug by strain interaction ($F_{3,74} = 0.76$, NS) were not significant (Fig. 3a).

For the time spent in the central square the strain effect was marginally significant ($F_{1,74} = 3.73$, $p > 0.05$) with the BIG1 mice tending to spend more time in the central square compared to the BIG2 mice. A significant drug effect ($F_{3,74} = 5.12$, $p < 0.005$) was primarily attributable to the 10 mg/kg dose, which increased the time spent in the central zone for BIG2 strains compared to the vehicle (Fig. 3b). The drug by strain interaction effect was not significant ($F_{3,74} = 0.47$, NS), which supports the view that the response at the 10 mg/kg dose in the BIG2 strain was minor and not replicated in the BIG1 strain.

For the number of line crossings there was a significant strain effect ($F_{1,74} = 19.61$, $p < 0.001$), which was due to the BIG1 strains having more line crossings when compared to the BIG2 strains (Fig. 3c). No significant drug effect ($F_{3,74} = 0.77$, NS) or drug by strain interaction ($F_{3,74} = 0.19$, NS) were found.

Discussion

Our results showed that a fluvoxamine treatment regimen for 15–17 days resulted in significant dose-dependent attenuation of compulsive-like nesting in BIG1 male mice with a

similar trend in the BIG2 male mice. This effect was observed following one hour of drug administration on the day of testing. Marble-burying behavior was dose-dependently reduced by fluvoxamine in both BIG1 and BIG2 strains. The dose response in nest-building and marble burying is in congruence with human studies where high doses of fluvoxamine have been found to be more effective for compulsive behaviors when compared to low doses (Fontenelle et al., 2007; Koran et al., 2010; Ordacgi et al., 2009). As fluvoxamine takes several weeks to months to become effective in the treatment of OCD patients (Fontenelle et al., 2007), we focused on the long-term effects of fluvoxamine. We tested the mice 15–17 days after daily injections, because in an earlier study another SSRI, i.e., fluoxetine, had significant effects after two weeks of chronic treatment in the drinking water (Greene-Schloesser et al., 2011). As we did not test the mice after the first injection of fluvoxamine, we cannot rule out acute effects. Fluvoxamine only had a significant effect on nest building in the first hour after administration and not at later time points, and therefore it is possible that the duration of fluvoxamine treatment had too short to see more persistent effects of this drug on compulsive-like nest building. It could also reflect that the response to fluvoxamine persists as long as the drug is active in the system. This is consistent with the refractory concept of OCD where patients have rebound of symptoms as soon as they are taken off the treatment regimen.

The BIG2 strains had a similar non-significant trend as the BIG1 strain in nest-building, which may have been due to the BIG2 males being less sensitive to fluvoxamine, having metabolized fluvoxamine more quickly, or having excreted it more rapidly than BIG1 males. Some OCD patients fail to respond in the typical dosage range used for clinical responders. Success of higher doses of SSRIs for non-responders has been observed (Declodt and Stein, 2010). However, a higher dose, though beneficial for OCD symptoms, can have severe side effects (Declodt and Stein, 2010; Bloch et al., 2010) thereby complicating the treatment strategies.

A previous study with the BIG1 mice has shown that a two-week fluoxetine (SSRI) treatment significantly attenuated nesting and marble burying behavior (Greene-Schloesser et al., 2011) in the BIG1 strain. This is the first time we have used both the BIG strains (BIG1 & BIG2) to compare the compulsive-like behavioral response to a first-line treatment, fluvoxamine. The results supported both nesting and marble-burying for assessment of compulsive-like behavior in our model. This is in contrast to the compulsive-like deer mouse model with spontaneous stereotypy, in which marble burying behavior was not associated with the stereotypy and did not respond to oral escitalopram treatment (Wolamrans et al., 2016b). So for deer mice, marble burying behavior did not appear to be a compulsive-like behavior. Interestingly, stereotypy and nest-building behavior also did not correlate, but 30% of the deer mice, irrespective of these two behaviors, showed unusually large nest-building behavior, which was attenuated by escitalopram (Wolamrans et al., 2016a). So, individual deer mice show different compulsive-like behaviors, i.e., stereotypy or nest building, but these were not correlated, while our BIG nest builders have a predictable compulsive-like phenotype showing both compulsive-like nesting and marble burying (Mitra et al., 2017) that can be attenuated with drugs used in OCD (Greene-Schloesser et al., 2011), as shown in this study. Therefore, it appears that compulsive-like behaviors are species specific and that

not all compulsive-like behaviors are genetically correlated or affected by the environment in similar ways.

Locomotor activity was measured in the open field test (Hale et al., 2008) and was not affected by fluvoxamine. This result replicates another study with fluvoxamine in mice in which repeated fluvoxamine administration attenuated marble burying but not locomotion or exploratory behavior (Ichimaru et al., 1995). Our results also conform to a previous study from our lab where fluoxetine treatment did not alter locomotor activity in the wheel running test (Greene-Schloesser et al., 2011). Overall, BIG2 mice showed higher levels of anxiety-like behaviors than BIG1 mice, measured as time to leave the central square and time spent in the center of the open field. The drug effect for time spent in central zone was mainly due to the 10 mg/kg of fluvoxamine increasing central-zone exploration in BIG2 males. However, owing to the lack of a significant strain effect and strain by drug interaction in central zone time, an overall effect of fluvoxamine on anxiety-like behavior cannot be established. Fluvoxamine also did not have any overall effect on locomotor activity. A prior study with mice has shown that fluvoxamine had no anxiolytic effects in different anxiety tests (Ichimaru et al., 1995). Our results show that behavioral responses to fluvoxamine, in the compulsive-like mice, were overall small or absent for anxiety-like measures, while strain differences were observed in anxiety-like behavior and locomotor activity. The strain effect may indicate genetic differences between the two BIG strains due to random genetic drift or founder effects (Bult and Lynch 1996, 2000).

While, fluvoxamine is well tolerated and used widely in the treatment of general anxiety disorder and OCD (Irons, 2005; Freeman et al., 1994; Mundo et al., 2000), not many studies have focused on looking at the effect of fluvoxamine in treating co-morbid anxiety in complex neuropsychiatric disorders (Nadeau et al., 2011). A study on the efficacy of fluvoxamine in treating autism spectrum disorder (ASD) and associated anxiety resulted in a poor response to treatment (Martin et al., 2003). The ego-dystonic and intrusive nature of obsessions differs largely from general anxiety (Langlois et al., 2000, 2000a). In addition to large heterogeneity and variability of anxiety in OCD, this makes it a non-significant indicator for the disorder (Nutt and Malizia, 2006). General anxiety disorders and panic disorders have a late onset when compared to OCD, which has a very early onset (Rosenbaum et al., 1997). In addition, neurocircuitry models proposed for anxiety and OCD differ. While anxiety and panic disorders have been linked to dysregulation of amygdala-ventromedial prefrontal cortex-hippocampus circuitry (Shin and Liberzon, 2010; Rauch et al., 2006), OCD is thought to occur due to abnormal fronto-striatal circuitry (Fitzgerald et al., 2005; Rauch et al., 2007; Greenberg et al., 2010). Other studies with our compulsive-like (BIG) and non-compulsive-like (SMALL) mice indicate that general anxiety appears to be separate from anxiety pertaining to compulsions (Greene-Schloesser et al., 2011; Mitra et al., 2017). This is corroborated by other studies where association of OCD with general anxiety is deemed controversial (Stein et al., 2010).

Currently a lack of consensus exists on the best treatment option for OCD patients (Pittenger et al., 2005). Significant relapse rates have been reported in clinical studies with SSRIs (Abramowitch et al., 2009). Medication differences have also been observed with these first-line treatments. One such example is that of comorbid tics in children with OCD, which

reduces the efficacy of SSRI treatment (March et al., 2007). It is however unclear if the same effect is persistent in adults (Lack, 2012). Among various factors, compulsive traits and associated co-morbid symptoms can result in non-responsiveness to treatments in the OCD condition (Koran, 2004). Studies have attributed a strong genetic link to the etiological heterogeneity of OCD (Nestadt et al., 2000; Pauls, 2010). These genes have also been thought to contribute to the disease severity and treatment resistance (Ozaki et al., 2003; Wendland et al., 2008). Quantitative genetic analysis indicates that nesting behavior is a highly polygenic trait (Bult and Lynch, 2000), which is consistent with OCD in humans likely being influenced by many genes (Hettema et al., 2001; Paul et al., 1999; Smoller, 2009; Stein, 2002). Hence, our mouse model selected for varied levels of compulsive-like behavior, i.e., nest-building, provides a critical insight into the role of genetic background and trait in influencing the efficacy of first-line treatments.

Conclusion

This study adds predictive validity to our mouse model of OCD because fluvoxamine, commonly used to treat OCD, dose-dependently decreased nest building behavior and marble burying behavior in the compulsive-like mice. Fluvoxamine had little to no effect on anxiety-like behaviors and locomotor activity, while one compulsive-like strain was more anxious than the other, which adds heterogeneity to our mouse model, similar to the heterogeneity seen in OCD.

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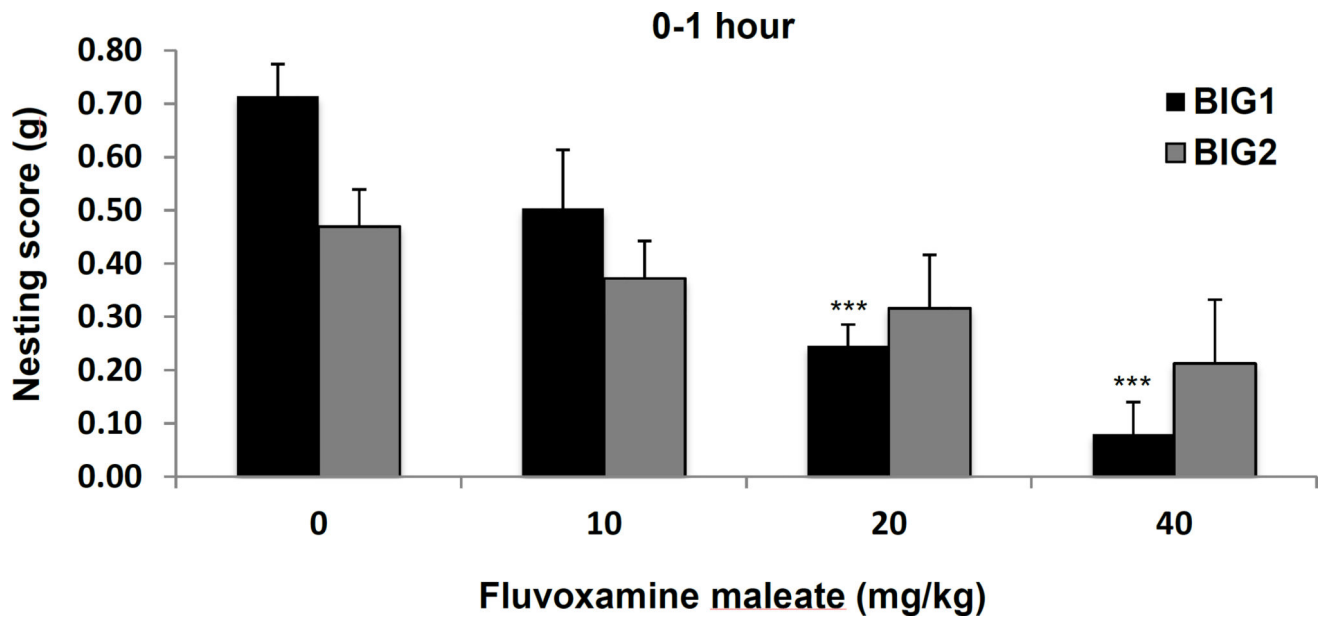


Figure 1.

Compulsive-like nest-building behavior of the BIG1 and BIG2 strains. The data represent the mean (+ SEM) for the nesting score, in grams, for the 0–1 hour period. ***($p < 0.001$) significant drug effect at 20 and 40 mg/kg relative to vehicle (0 mg/kg) in the BIG1 strain. 0 mg/kg, $n=11$ and $n=11$; 10 mg/kg, $n=7$ and $n=12$; 20 mg/kg, $n=10$ and $n=8$; 40 mg/kg, $n=6$ and $n=9$, for BIG1 and BIG2, respectively.

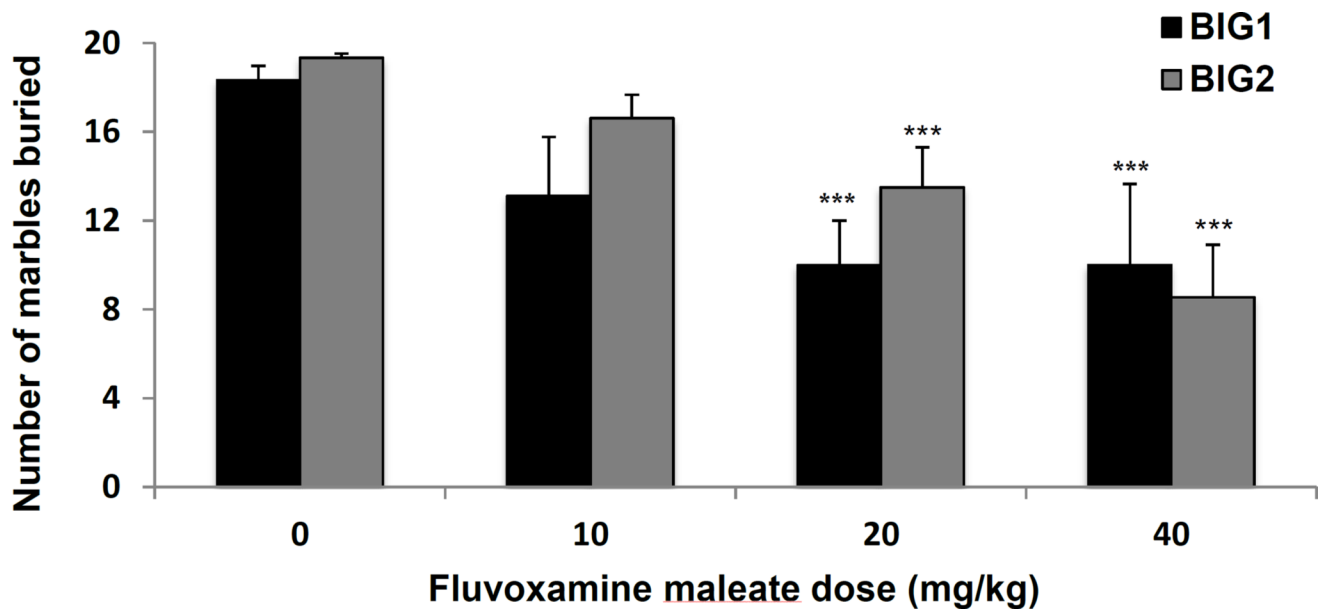
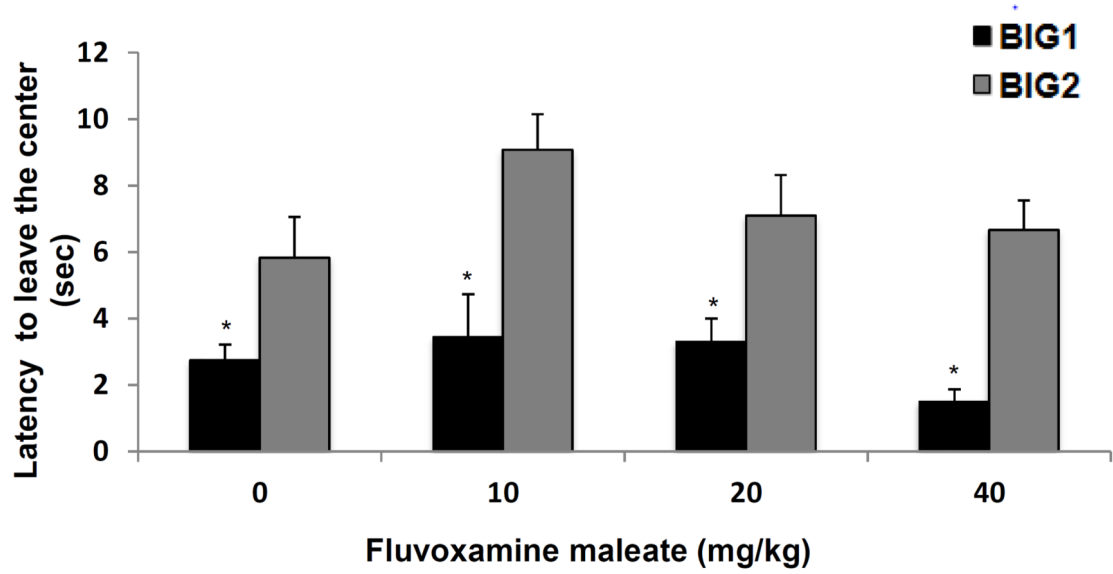
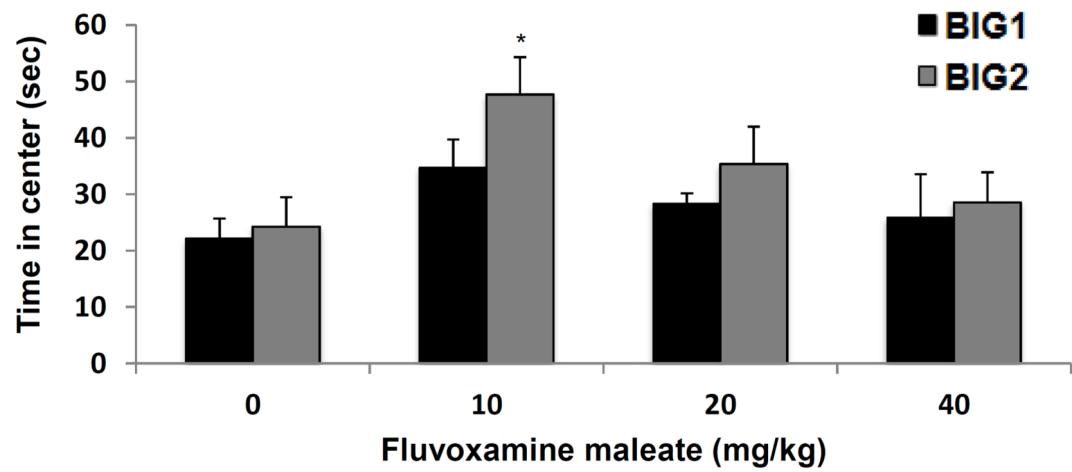


Figure 2.

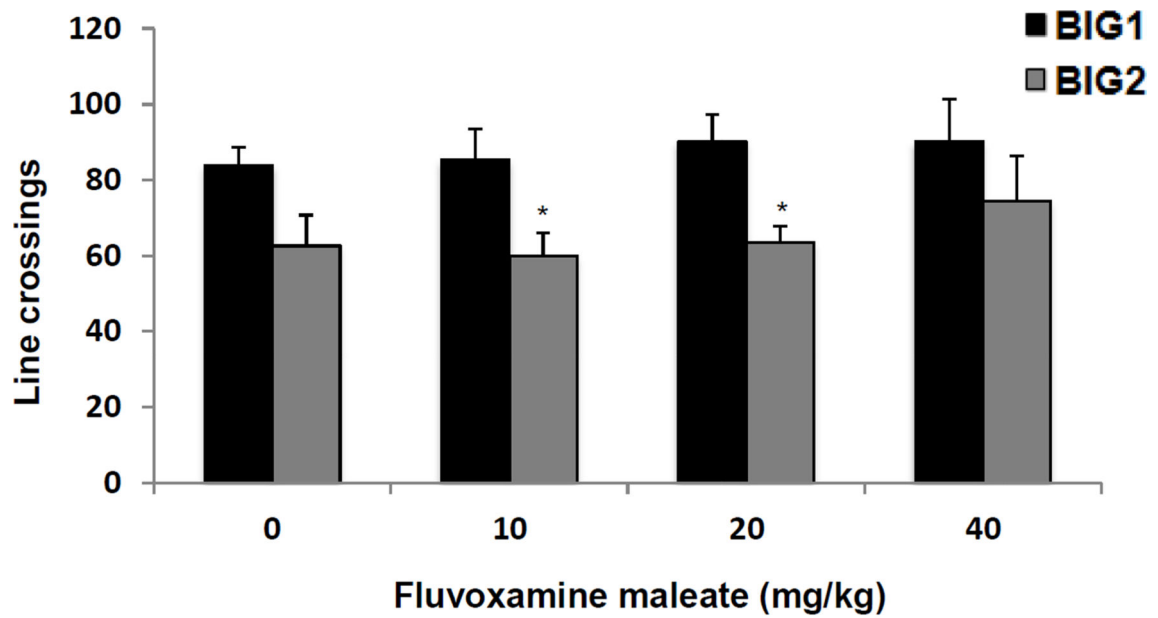
Compulsive-like marble burying behavior of the BIG1 and BIG2 strains. The data represent the mean (+ SEM) for total number of marbles buried. ***($p < 0.001$) indicates significant drug effect at 20 and 40 mg/kg relative to vehicle (0 mg/kg) in both BIG1 and BIG2 strains. 0 mg/kg, $n=11$ and $n=11$; 10 mg/kg, $n=7$ and $n=12$; 20 mg/kg, $n=10$ and $n=8$; 40 mg/kg, $n=6$ and $n=9$ for BIG1 and BIG2, respectively.



(a)



(b)



(c)

Figure 3.

Anxiety-like open field behavior of the BIG1 and BIG2 strains. The data represent the mean (+ SEM) for (a) latency to leave the central square, *($p < 0.05$) indicates significant strain differences between BIG1 and BIG2 males in each treatment group; (b) the time spent in the central square, *($p < 0.05$) indicates significant drug effect at 10 mg/kg relative to vehicle (0 mg/kg) in the BIG2 strain; and (c) the total number of line crossings, *($p < 0.05$) indicates strain differences between BIG1 and BIG2 for the 10 and 20 mg/kg treatment groups. 0 mg/kg, $n = 11$ and $n = 11$; 10 mg/kg, $n = 7$ and $n = 12$; 20 mg/kg, $n = 10$ and $n = 8$; 40 mg/kg, $n = 6$ and $n = 9$ for BIG1 and BIG2, respectively.