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Predictive Validity of a Non-Induced Mouse Model of Compulsive-Like Behavior

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Abstract

A key to advancing the understanding of obsessive-compulsive disorder (OCD)-like symptoms is the development of spontaneous animal models. Over 55 generations of bidirectional selection for nest-building behavior in house mice, *Mus musculus*, resulted in a 40-fold difference in the amount of cotton used for a nest in high (BIG) and low (SMALL) selected lines. The nesting behavior of BIG mice appears to be compulsive-like and has initial face validity as an animal model for OCD in humans. Compulsive-like digging behavior was assessed; BIG male mice buried about three times as many marbles as SMALL male mice, strengthening face validity. Using the open field and elevated plus maze, SMALL male mice showed higher levels of anxiety/fear-like behavior than BIG male mice, indicating that compulsive-like and not anxiety-like behavior was measured. To establish predictive validity, chronic (4 weeks) oral administration of fluoxetine (30, 50 and 100 mg/kg/day) and clomipramine (80 mg/kg/day), both effective in treating OCD, significantly reduced compulsive-like nest-building behavior in BIG male mice. Compulsive-like digging behavior was also significantly reduced by chronic oral fluoxetine (30 and 80 mg/kg/day) treatment in BIG male mice. General locomotor activity was not affected by chronic oral fluoxetine (30 and 80 mg/kg/day) treatment; chronic oral treatment with desipramine (30 mg/kg/day), an antidepressant not effective in treating OCD, had no effect on nesting behavior of BIG male mice, strengthening predictive validity. Together, the results indicate that these mice have good face and predictive validity as a non-induced mouse model of compulsive-like behavior relevant to OCD.

Keywords

Obsessive-Compulsive Disorder; Nesting; Mouse Model; Fluoxetine; Clomipramine; Chronic Oral Administration

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1. Introduction

Obsessive-compulsive disorder (OCD) is a debilitating psychiatric condition characterized by intrusive and persistent thoughts (obsessions) and repetitive behaviors (compulsions) (DSM-IV-TR, American Psychiatric Association, 2000). The neural mechanisms that control OCD are poorly understood partly due to the relatively small number of animal models available that reveal consistent and spontaneous (non drug-induced, non behaviorally-induced) differences in compulsive-like behaviors, including somersaulting, jumping, and pattern running, in deer mice (Korff *et al.*, 2008, 2009) and maternal nest building in rabbits (Hoffman and Morales, 2009). Other proposed spontaneous animal models of compulsive-like behaviors include barbering in mice (e.g., Garner *et al.*, 2004) and acral lick dermatitis in dogs (e.g., Wynchank and Berk, 1998), but these models lack consistency and/or predictability. Mice with induced single gene mutations with spontaneous compulsive-like behaviors are becoming more prevalent and will contribute to the understanding of particular neural pathways that contribute to compulsive-like behaviors (Boulougouris *et al.*, 2009; Feusner *et al.*, 2009; Wang *et al.*, 2009).

Specific requirements for the development of successful animal models include face, predictive and construct validity. Face validity of the animal model is comprised of homologous symptomology to human OCD patients and objectively measurable behavioral changes (Boulougouris *et al.*, 2009; Korff and Harvey, 2006; McKinney and Bunney, 1969; Overall, 2000; Wilner, 1991). Over 55 generations of bidirectional artificial selection in house mice resulted in a spontaneous and consistent 40-fold difference between big (BIG) and small (SMALL) nest-building house mice, *Mus musculus*, in the amount of cotton used for a nest (Bult and Lynch, 1996, 1997, 2000; Lynch, 1980). Nest-building behavior is characterized by rapid and repeated movements of the front legs, paws and mouth to pull in cotton through the metal bars of the cage top and is a natural house mouse behavior (Lynch, 1992), which provides initial face validity of the compulsive-like nest-building mouse model relevant to OCD. To establish additional face validity of a compulsive-like phenotype, the BIG mice were tested for marble burying as a measure of compulsive-like digging behavior (Gyertyán, 1995; Njung'e and Handley, 1991; Takeuchi *et al.*, 2002). Marble-burying behavior has also been used as a measure of anxiety (Borsini *et al.*, 2002; Li *et al.*, 2006). To determine whether marble burying reflected compulsive-like compared to anxiety-like behaviors, the mice were tested for performance in an open field and elevated plus-maze (Meerlo *et al.*, 1999; Pellow, *et al.*, 1985).

The main objective of this paper is to establish predictive validity by assessing if treatment responses in BIG male mice are similar to those seen in human OCD patients (Overall, 2000). Medications targeting the serotonin system, such as fluoxetine and clomipramine, rank as the most popular and effective forms of pharmacological treatment of OCD symptoms in humans (Goodman *et al.*, 1990; Greist, 1998; Jenike, 2004; Micallef and Blin, 2001; Stein, 2002). In contrast, treatment with desipramine, a selective noradrenergic re-uptake inhibitor, has been shown to be consistently ineffective (Goodman *et al.*, 1990; Greist, 1998; Hoehn-Saric, *et al.*, 2000; Micallef and Blin, 2001). Hence, effects of fluoxetine on nesting and marble burying were elucidated to establish predictive validity for our mouse model for OCD. Daily wheel-running activity was used to control for fluoxetine effects on general activity levels. Effects of clomipramine and desipramine on nesting behavior of BIG male mice were assessed to strengthen predictive validity.

2. Methods

2.1. Subjects

2.1.1. For Assessment of Compulsive-Like Behaviors, Wheel-Running Activity, and Drug Effects—BIG and SMALL male house mice, *Mus musculus*, were raised on wood shavings in polypropylene cages (27×17×12 cm) under a 12:12 light-dark cycle at 22±1°C. Pups were weaned at 19-21 days of age and housed with same-sex littermates until the start of the experiment. Food (Purina Mills, Lab Diet Mouse Diet #5015, St. Louis, MO) and water were available *ad libitum*. All animals were approximately 2 months of age at the start of each experiment. The University of Alaska Fairbanks Institutional Animal Care and Use Committee approved the animal care and experimental procedures (IACUC #02-57; IACUC #05-64).

2.1.2. For Assessment of Anxiety-Like Behaviors—BIG and SMALL male house mice, *Mus musculus*, were individually housed (cage size: 18 × 12 × 13 cm). Animals were maintained in a 12:12 hr light-dark schedule (lights on at 08:00) in a climate room at 20 ± 0.5 °C. Pups were weaned at 19-21 days of age and housed with same-sex littermates until the start of the experiment. Food (Hope Farms® mouse pellets, Woerden, Netherlands) and water were available *ad libitum*. All animals used in these studies were between 3 to 5 months of age. Principles of Laboratory Animal Care (NIH publication No. 86-23, revised 1985) were followed, and the Animal Experimentation Committee of the University of Groningen approved the experiments (Dec. No. 2091 & 2093).

2.2. Compulsive-Like Behaviors

2.2.1. Marble-Burying Behavior—The marble-burying test has been used as an effective model of compulsive-like behaviors in mice (Njung'e and Handley, 1991; Gyertyán, 1995; Takeuchi *et al.*, 2002). During the test period, BIG and SMALL male mice were placed individually in a polypropylene cage (37×21×14 cm) containing 20 glass marbles (10 mm in diameter) evenly spaced on 5 cm deep sawdust without access to food or water for 20 min. Testing cages were located in a room separate from the housing room. The number of marbles that were at least 2/3 buried within a 20-min period quantified compulsive-like digging activity. After the 20-min test, the animals were returned to their home cages. This test was performed once a week (on the same day at the same time), by two independent observers blind to the subjects' nesting phenotype and treatment condition. The correlation coefficients, as measures of the inter-rater reliability, for five different rater pairs were 0.878, 0.949, 0.959, 0.970 and 0.985.

2.2.2. Nest-building Behavior—Nest-building behavior was assessed as a novel compulsive-like phenotype in mice. BIG and SMALL male mice were provided with a pre-weighed roll of cotton (Mountain Mist cotton batting, Troy, Inc., Chicago, Illinois) in the cage-top food hopper. Every 24 hours, for 4 consecutive days, the cotton roll was weighed and put back after the nest had been removed. Extra cotton was added when necessary. On the fifth day the cotton roll was removed and weighed, and the mice were placed in clean cages with wood shavings and no cotton nesting material. The total nesting score was defined as the amount of cotton used over the 4-day testing period (Bult & Lynch, 1996, 1997, 2000). Nesting behavior is quantified by the total nesting score. This procedure was followed every week for the duration of the experiments.

2.3. Wheel-Running Behavior

Wheel-running behavior was used to assess general activity levels as a control. BIG male mice were individually housed in cages equipped with running wheels. Wheel-running activity was measured using 24.2-cm diameter Nalgene running wheels mounted in

polycarbonate cages (21×42×20 cm) equipped with a magnetic switch and wood shaving bedding. The number of wheel revolutions was continuously recorded in 5-min bins by computer with the VitalView data collection system (Mini-Mitter Co., Inc., Bend, Oregon). Wheel-running activity was quantified following standard protocols (Amy *et al.*, 2000; Castillo *et al.*, 2004, 2005; Hochstetler *et al.*, 2004; Yan *et al.*, 2003).

2.4. Anxiety-Like Behaviors

2.4.1. Open Field Behavior—Open field behavior was used to assess anxiety-like behaviors in mice (Meerlo *et al.*, 1999). BIG and SMALL male mice (n=10) were brought to the testing room 24 hrs before the open field test was performed. All tests took place between 13:00 and 15:00. The open field (82 cm wide, 32 cm deep) consisted of an inner and outer zone (17 cm distance from the edge). A 40-Watt light bulb was placed above the open field at a height of 1.4 m. The animals were placed in the center of the open field for 3 min and their behavior was video taped and analyzed with the aid of EthoVision (Noldus, Wageningen, Netherlands). The time taken to leave the center and reach the outer zones (“latency”), and the times spent in the outer and inner zone were measured. In addition, the number of rears (standing on hind legs) and the total distance traveled through the open field by the subjects were recorded. The open field was cleaned before each test.

2.4.2. Elevated Plus-Maze Behavior—To test whether fear-like behavior in the SMALL male mice inhibited exploratory behavior and the rapid adjustment to a new and unfamiliar environment, an elevated plus-maze test was performed on all nest-builders previously tested in the open field (Belzung and Griebel, 2001; Parks *et al.*, 1998; Pellow *et al.*, 1985). BIG and SMALL male mice (n=10) used in the open field test were taken to another testing room 3-7 days before the elevated plus maze test. The plus-maze was made of grey Perspex and elevated to a height of 75 cm. It consisted of two open arms (30 × 5 cm, with 4 mm high ledges) and two enclosed arms (30 × 5 cm, with a closed roof). All tests took place between 13:00 and 15:00 under dim red light. Each mouse was placed in the central square (5 × 5 cm) facing an open arm, and allowed to explore the maze for 5 min. The times spent on the open arms, closed arms and in the center were measured as well as the number of entries into a closed arm, into an open arm, and the total number of entries (Pellow *et al.*, 1985). An entry was defined as at least three of the four paws being on the arm. The maze was cleaned before each test. The mice were not used for any additional experiments.

2.4.2.1. Experimentally-Naïve and Experimentally-Experienced Individuals: The following study was done as a control measure to determine if any degree of fear-like behavior in the elevated plus maze may have been influenced by the animals’ experience with previous exposure to an unfamiliar environment when tested for open field behavior. BIG and SMALL male mice that were experimentally-naïve (n = 5 per group) were tested in the elevated plus-maze as described above and compared to experimentally-experienced individuals. The mice were not used for any additional experiments.

2.5. Pharmacological treatments

In order to provide better validity, the following experiments used chronic oral drug delivery via drinking water (Boscarino and Parfitt, 2002; Kobayashi *et al.*, 2008; Dulawa *et al.*, 2004). Oral drug delivery provides advantages, such as a similar delivery route as seen in human clinical settings, as well as reduced handling and injection stress on the mice. In addition, drug delivery in the drinking water also allowed for a more sustained level of drug delivery throughout the day compared to daily injections. However, the oral delivery did not allow for the exact determination of the active dose the animals received (all doses were approximately within 5% of dose based on thorough measurement of fluid consumed),

although relative comparisons among drug doses were appropriate. Water consumption levels were not altered by the inclusion of the drugs in the water as compared to pre-drug baseline consumption levels (data not shown).

All mice were tested for drinking behavior for one week prior to preliminary behavioral measurements. Average daily water consumption was used to calculate the drug concentration of fluoxetine, clomipramine and desipramine. Sucrose (2.9 g/L) was added to the tap water to increase palatability of clomipramine and desipramine. The fluoxetine suspension used contained sucrose (2.9 g/L). Drugs were added to the drinking water and available *ad libitum*. The contents of the bottles were changed every other day (Kobayashi *et al.*, 2008) to ensure that active drugs were administered. Water consumption was measured daily to ensure appropriate dosage and hydration and body weight was measured weekly as an indicator of the animals' health. No significant change in water consumption or body weight was noted with the addition of the drugs in any of the following experiments (data not shown), except for water consumption in the wheel running experiment as described in section 2.5.1.3.

2.5.1. Fluoxetine

2.5.1.1. Effects on Nest-Building Behavior: BIG male mice were orally treated with fluoxetine (5, 10, 30, 50, or 100 mg/kg/day, n=11 or 12 per group). The fluoxetine doses were determined following preliminary studies with BIG male mice using chronic oral doses of 5, 15, and 30 mg/kg/day for 4-8 weeks, in which the 30 mg/kg/day reduced nest-building behavior significantly (data not shown). The high oral dose of 100 mg/kg/day was close to the upper tolerability level as it was too high for mice running in wheels (section 2.5.1.3). Control mice were given sucrose (2.9 g/L) in tap water. These were separate groups of individuals that were only used for this experiment. Mice were tested for baseline nest-building behavior and water consumption (tap water) for 4 weeks prior to drug administration. Nesting behavior was tested during drug treatment for 4 weeks, followed by an additional 4 weeks post-drug treatment to test for drug washout effects.

2.5.1.2. Effects on Marble-Burying Behavior: Marble burying was tested as an additional compulsive-like behavioral measurement to further add face validity for the compulsive-like nest-building mouse model. BIG male mice (n=11 or 12 per group) were orally treated with fluoxetine (30 or 80 mg/kg/day, to allow comparison to the low and high doses used to assess the effects on wheel running behavior and to the low and high doses that were effective in reducing nest-building behavior) or sucrose vehicle (2.9 g/L, in tap water). These were separate groups of animals that were only used for this experiment. Mice were tested once a week for 3 weeks to obtain baseline marble-burying behavior. Marble burying was assessed once a week during the 4-week treatment period and 4 weeks post-treatment to test for drug washout effects.

2.5.1.3. Effects on Wheel-Running Behavior: Wheel-running behavior was used to determine if changes in nest-building and marble-burying behavior were due to a general decrease in locomotor activity. BIG male mice (n=11 or 12 per group) were orally treated with 30 or 80 mg/kg/day of fluoxetine to allow comparison to the low and high doses that were effective in reducing nest-building behavior. Control mice (n=11) were given sucrose (2.9 g/L) in tap water. These were separate groups of animals that were only used for this experiment. Locomotor behavior was assessed for 5 weeks prior, 8 weeks during and 4 weeks post-drug treatment to test for drug washout effects, using average daily wheel-running activity levels per 5-min bin. After administration of 100 mg/kg/day of fluoxetine, the mice decreased their drinking behavior significantly within a couple of days and the dose was immediately reduced to 80 mg/kg/day after which the mice resumed normal drinking

behavior. The larger level of activity while running in the wheel (distances of up to 10 km/day), compared to nest-building behavior, may have interacted with fluoxetine to decrease tolerability. Consequently, the high fluoxetine dose in the marble-burying test was set at 80 mg/kg/day (section 2.5.1.2) to allow for direct comparison.

2.5.2. Effects of Clomipramine on Nest-Building Behavior—BIG male mice were divided into two drug groups (n=16 per group): clomipramine (20 mg/kg/day; Sigma Aldrich Inc., St Louis, MO) and control (tap water containing sucrose, 2.9 g/L). After 8 weeks, drug doses were increased (drug groups were split, resulting in n=7 or 8 per group): one 40 mg/kg/day group and another 80 mg/kg/day group. These doses were adapted from oral clomipramine doses used in rabbits (Boscarino and Parfitt, 2002). The mice were tested for baseline nest-building behavior for 3 weeks prior to drug treatment. Nest-building behavior was tested for 8 (20 mg/kg/day) + 4 (40 or 80 mg/kg/day) weeks during drug treatment and then for 4 additional weeks post-treatment to test for drug washout effects.

2.5.3. Effects of Desipramine on Nest-Building Behavior—BIG male mice (n=11 or 12 per group) were orally treated with desipramine (Sigma Aldrich Inc., St Louis, MO) (30 mg/kg/day), fluoxetine (50 mg/kg/day) or sucrose vehicle (2.9 g/L, in tap water). These were separate groups of animals that were only used for this experiment. The 30 mg/kg/day dose was chosen as it is the highest intraperitoneal (i.p.) dose used in other behavioral studies in rats and mice (Li *et al.*, 2006; Rosenzweig-Lipson *et al.*, 2007; Weber *et al.*, 2009), while behavioral effects of desipramine in mice were detected at doses as low as 1-2 mg/kg i.p. (Bourin *et al.*, 1998; David *et al.*, 2003). In addition, acute oral desipramine doses in mice ranged from 15-60 mg/kg (Ichimaru *et al.*, 1995), but because of the chronic treatment in this experiment the 30 mg/kg/day was chosen for safety. Nest-building behavior was recorded for 3 weeks prior to drug treatment, 4 weeks during drug treatment and 4 weeks post-treatment to test for drug washout effects.

2.6. Statistical Analyses

2.6.1. For Assessment of Anxiety-Like Behaviors—A Mann Whitney U-test (MWU) or a Student's t-test was used for statistical analysis. In addition, in the naïve versus experienced experiment, an ANOVA for repeated measures was performed, in which the mouse line was the between-subject factor and treatment (naïve versus experienced) the within-subject factor. We used simple contrast, comparing values of each single block with the first, to determine interaction effects. Finally, a post-hoc Student's t-test analysis at block 1 was done to compare the initial performance between the lines. A probability level of $p < 0.05$ was used as an index of statistical significance in all cases. All tests were applied two-tailed, and all data are presented as mean $\pm$ standard error of the mean ($\pm$ SEM).

2.6.2. For Assessment of Compulsive-Like Behaviors, Wheel-Running Activity, and Drug Effects—Nest-building, wheel-running and marble-burying behavioral data were analyzed by repeated-measures ANOVA with time (1-week periods for the entire experiment) and drug or drug dose effects. The General Linear Models (GLM) procedure also included Time x Drug interaction effects. If significant effects were found, pair-wise differences were tested for significance using the Tukey Studentized Range Test (Sokal and Rohlf, 1981). All statistics were done using SAS software (Version 9.1.3, Cary, NC).

3. Results

All drugs used (fluoxetine, clomipramine and desipramine) had no significant effect on body weight of the treatment groups as compared to controls (data not shown).

3.1. Face Validity of a Compulsive-Like Phenotype

3.1.1. Marble-Burying Behavior in Nest-Building Mouse Lines—Marble-burying behavior differed significantly between BIG and SMALL male mice ($F_{3,20}=48.02$, $p<0.0001$) and BIG mice buried about three times as many marbles (16 on average) as SMALL mice (5 on average) (Fig. 1).

3.1.2. Anxiety-Like Behaviors in Nest-Building Mouse Lines

3.1.2.1. Open Field Behavior: Performance in the open field differed significantly between BIG and SMALL male mice in all measures. SMALL male mice showed more inhibited exploratory activity and risk assessment behavior in the open field. They typically started exploration of the inner zone of the open field by rotating around their hind paws, without moving their hind paws from the center area where they were initially placed. Total exploratory activity, indicated by the distance traveled in the open field, was two-fold and significantly longer in BIG than SMALL mice (Table 1; $p<0.0001$; Student's t-test). Time taken before leaving the center of the open field and to explore the outer zone was longer in SMALL than in BIG mice. SMALL mice took almost 10 times longer than BIG mice to start exploring the outer zone (Table 1; $p<0.002$; MWU). The times spent in the inner and outer zones differed significantly between the lines (Table 1; $p<0.05$ and $p<0.01$, respectively; MWU).

Rearing frequency in the outer zone (peripheral rearing) differed significantly between the lines, with BIG mice rearing approximately three times more often than SMALL mice (Table 1; $p<0.01$; MWU). Center rearing in the outer zone was rarely seen and was only observed in BIG mice. The time spent in center rearing differed 4-fold and significantly between the two lines (Table 1, $p<0.05$; MWU).

3.1.2.2. Elevated Plus-Maze Performance: General activity (indicated by the number of entries into open and closed arms as well as the total entries) was significantly higher in the experimentally-experienced BIG compared to SMALL male mice. The time spent on open or closed arms also differed between the lines. The experimentally-experienced SMALL mice spent significantly less time on the open arm ($p<0.01$; MWU), and significantly more time in the closed arm ($p<0.05$; MWU) than the experimentally-experienced BIG male mice. Experimentally-experienced SMALL mice showed enhanced exploratory behavior, indicated by the significantly higher number of arm entries into the open arm ($p<0.04$; MWU) and total arm entries ($p<0.04$; MWU) compared to the less experienced SMALL mice (Table 2). No such differences were observed for BIG male mice (Table 2). Furthermore, experimentally-naïve BIG and SMALL male mice differed significantly for all elevated plus maze performance measures ($p<0.05$; MWU). Experimentally-naïve BIG mice had more total arm entries, more closed arm entries, more open arm entries, spent more time in the center and in the open arms, and spent less time in the closed arms than the experimentally-naïve SMALL mice (Table 2).

The percent of time spent in open arms (of total time spent in arms) provides an additional measure of anxiety-like behavior (calculated from data obtained from Table 2) (Belzung and Griebel, 2001; Parks *et al.*, 1998; Pellow *et al.*, 1985). BIG mice spent 24.0 ± 4.2 % of their time in the open arms, but SMALL mice spent significantly less time there (7.7 ± 2.4 %; $p<0.008$; MWU). No significant difference was found between experimentally-experienced and experimentally-naïve BIG or SMALL mice.

3.2. Predictive Validity of a Compulsive-Like Phenotype

3.2.1. Fluoxetine Effects on Nest-Building Behavior—Fluoxetine significantly reduced nest-building behavior in BIG male mice. The 100 mg/kg dose showed the largest

decrease in nesting behavior (< 40% of pre-drug levels; Fig. 2), followed by smaller decreases in animals exposed to 50 mg/kg and 30 mg/kg, respectively (repeated-measures ANOVA for Drug effect: $F_{5,62}=2.99$, $p<0.02$; Time effect: $F_{9,558}=29.38$, $p<0.0001$; Drug x Time interaction effect: $F_{45,558}=4.92$, $p<0.0001$). The 5 mg/kg and 10 mg/kg doses had no effect on the level of nesting behavior compared to the control group. All drug-treated mice returned to baseline levels by week 3 of no drug treatment (Fig. 2).

3.2.2. Fluoxetine Effects on Marble-Burying Behavior—BIG male mice significantly reduced their marble-burying behavior by approximately half of their pre-drug digging scores after both the 30 and 80 mg/kg doses (Fig. 3; repeated-measures ANOVA for Drug effect: $F_{2,32}=2.99$, $p<0.04$; Time effect: $F_{10,320}=17.38$, $p<0.0001$; Drug x Time interaction effect: $F_{20,320}=3.78$, $p<0.0001$). The two doses did not differ significantly. All drug-treated mice returned to baseline levels by week 1 of no drug treatment (Fig. 3).

3.2.3. Fluoxetine Effects on Wheel Running Activity—Fluoxetine did not significantly change wheel-running activity of BIG male mice (Fig. 4; repeated measures ANOVA for Drug effect: $F_{1,40}=0.21$, $p>0.50$). All drug groups (control, 30 and 80 mg/kg fluoxetine) reduced their average locomotor activity level by approximately 50% of pre-drug values towards the end of the drug treatment period (repeated measures ANOVA for Time effect (week): $F_{1,40}=13.28$, $p<0.0008$).

3.2.4. Clomipramine Effects on Nest-Building Behavior—The BIG male mice that received 80 mg/kg/day clomipramine had a significant reduction in nest-building levels after drug treatment (Fig. 5A; repeated measures ANOVA for Drug effect: $F_{1,14}=7.53$, $p<0.02$; respectively). After drug treatment ended, nesting scores returned to control levels after 2 weeks. The lower drug doses of 20 and 40 mg/kg/day had no significant effects (Fig. 5B).

3.2.5. Desipramine Effects on Nest-Building Behavior—Desipramine-treated BIG male mice had no significant difference in cotton usage from the control group (Fig. 6; $F_{1,31}=0.43$; $p>0.50$). A significant drug (comparing control, desipramine, fluoxetine treatment groups) effect ($F_{2,46}=7.74$; $p<0.002$), a significant time (week) effect ($F_{10,460}=28.75$; $p<0.0001$), and a significant drug by time interaction effect ($F_{20,460}=5.58$; $p<0.0001$) were found. Fluoxetine treated mice significantly decreased cotton usage as compared to control ($F_{1,30}=16.01$; $p<0.0005$) with significant time (week) ($F_{10,300}=20.51$; $p<0.0001$) and drug by time (week) interaction ($F_{10,300}=10.51$; $p<0.0001$) effects. All drug-treated mice returned to baseline levels by week 2 of post-drug treatment (Fig. 6).

4. Discussion

In addition to compulsive-like nest building, BIG male mice also displayed compulsive-like digging behavior, as evidenced by the number of marbles buried. The marble-burying behavior has been used to assess compulsive-like behaviors in mice using digging, a non-fear induced behavior (Njung'e and Handley; 1991). However, Borsini *et al.* (2002) have suggested that marble burying may be a model for anxiety. The present data obtained from two behavioral tests commonly used to assess anxiety/fear-like behavior in rodents, i.e., open field and elevated plus maze, suggested otherwise in the nesting mice. These data revealed a consistent difference between the BIG and SMALL male mice in these anxiety-like measures. In general, SMALL mice displayed a more anxiety-like inhibition of behaviors in response to a novel environment, while the BIG mice had consistently higher general activity levels. The behavior shown by SMALL mice in the open field and elevated plus-maze, which can be interpreted as (predatory) risk assessment behavior and movement inhibition, clearly indicated higher levels of anxiety/fear-like behavior (Belzung and Griebel, 2001; Parks *et al.*, 1998; Pellow *et al.*, 1985). Taken together, these data support

marble-burying behavior as a test for compulsive-like behaviors in these mice, not for anxiety. This provides further support for the face validity of these mice as a potential animal model of OCD with a focus on compulsive-like behavior as obsessions are very difficult to measure in mice.

Chronic oral fluoxetine treatment dose-dependently reduced compulsive-like nest-building behavior in BIG male mice. Marble burying was also reduced after fluoxetine treatment but a dose-response was not observed as the 30 and 80 mg/kg/day doses showed similar decreases. Importantly, the administration of fluoxetine did not cause a decrease in general locomotor behavior, as assessed by wheel-running activity. All BIG male mice (no-drug control and drug groups) in the wheel-running experiment showed a reduction in average locomotor activity towards the end of the drug treatment period, which may have been due to an age effect or uncontrolled environmental factors such as humidity (Lynch, 1992). The maximum dose of fluoxetine that we could test while the animals were using the wheels was 80 mg/kg/day, because the 100 mg/kg/day dose resulted in a significant decrease in water drinking behavior. Although we do not know whether the 100 mg/kg/day dose would have reduced activity levels, the 30 and 50 mg/kg/day fluoxetine doses decreased nest-building behavior significantly and the 30 and 80 mg/kg/day fluoxetine doses decreased marble burying significantly, while these doses did not decrease wheel-running levels significantly. Therefore, the effects of fluoxetine appear to be specific to the compulsive-like behaviors. Combined, these findings support that the nest-building phenotype of the BIG mice has good predictive validity for modeling compulsive disorders, such as OCD, in humans.

Fluoxetine is a commonly prescribed serotonin re-uptake inhibitor for the treatment of OCD symptoms in humans (Goodman *et al.*, 1990; Greist, 1998; Micallef and Blin, 2001). The latency to peak effect found in the present study, of approximately 3 to 4 weeks, is similar to the delayed response of SSRIs in human OCD patients (Man, *et al.*, 2004). Chronic oral fluoxetine administration also decreased nest-building behavior of BIG male mice in a dose-dependent manner. The route of drug administration differs from most other animal models, but has been used successfully with doses similar to those used in this study (Kobayashi *et al.*, 2008; Dulawa *et al.*, 2004). Using an oral delivery of fluoxetine provides advantages as described in section 2.5. However, due to efficiency in absorption and excretion, bioavailability of fluoxetine after oral administration is much lower than through other routes (Caccia *et al.*, 1990). For example, a dose of 10 mg/kg intravenously (IV) produces a maximum plasma concentration (C_{max}) of 3.8 μ M, whereas the same dose through oral administration results in a C_{max} of 0.4 μ M, or about 10% of the IV route. Hence, oral doses need to be higher than those administered through more direct routes to compensate for the hepatic first-pass effect (Caccia *et al.*, 1990). This first-pass metabolism of fluoxetine by the liver is likely subject to transient saturation with increasing doses, thereby producing a complex, nonlinear pharmacokinetic profile (Caccia *et al.*, 1990; Altamura *et al.*, 1994). Furthermore, these pharmacological properties of fluoxetine are manifested differently in rodents and in humans (Altamura *et al.*, 1994). Hence, care should be taken when extrapolating animal dose-response models using fluoxetine to clinical applications. Future studies need to be conducted to determine the plasma and brain concentrations of fluoxetine and its metabolites using oral administration in the nesting mice.

Chronic oral clomipramine significantly reduced nest-building behavior in the BIG mice, and as clomipramine is another common drug used to treat OCD (Goodman *et al.*, 1990; Greist, 1998; Jenike, 2004; Micallef and Blin, 2001; Stein, 2002), this finding adds further predictive validity to the nesting mouse model. Interestingly, the lowest effective clomipramine dose of 80 mg/kg/day was higher than the lowest effective dose of 30 mg/kg/day for fluoxetine. This is in general agreement with human use of these drugs in which recommended fluoxetine doses (40-80 mg/day) are about one-third of those of clomipramine

(150-250 mg/day) (Dell'Osso *et al.*, 2007). In our experiment, we probably used relatively low clomipramine doses and might have obtained a more complete dose response by using higher doses. The bioavailability of orally administered clomipramine in mice is not known, but the effectiveness of orally administration is supported by a study in rabbits in which clomipramine was administered in the drinking water for 14 consecutive days at doses of 40 and 60 mg/kg/day. Both doses inhibited adult male sexual behavior by significantly reducing the number of ejaculations (Boscarino and Parfitt, 2002).

As a negative control, chronic oral desipramine, a drug shown not to be effective in the treatment of OCD in humans (Hoehn-Saric *et al.*, 2000), did not significantly alter nesting behavior of the BIG mice at a dose of 30 mg/kg/day, which may strengthen the predictive validity of the nesting mouse model. Alternatively, the desipramine dose may not have been high enough to determine whether it had an effect on nest-building behavior in the BIG mice. The 30 mg/kg/day dose was the highest i.p. dose used in other behavioral studies (Li *et al.*, 2006; Rosenzweig-Lipson *et al.*, 2007; Weber *et al.*, 2009), but the oral administration most likely reduced the effective dose significantly. Nevertheless, effects of desipramine on mice in the forced swimming test, a model for depression, were detected at doses as low as 1-2 mg/kg i.p. (Bourin *et al.*, 1998; David *et al.*, 2003). Acute oral desipramine doses in mice, ranging from 15-60 mg/kg, did not affect marble-burying behavior, while acute oral clomipramine (60 mg/kg) significantly depressed this behavior (Ichimaru *et al.*, 1995). This finding is qualitatively similar to the effects of chronic oral administration of these drugs on nest-building behavior as described.

The nest-building behavior exhibited by our mice has a genetic component in which approximately 30% of the variation among individuals in the expression of the behavior can be attributed to additive genetic factors, while approximately 70% of the variation is mostly due to environmental factors (Bult and Lynch, 2000; Lynch, 1980). OCD in humans also appears to have a genetic component as well as environmental components (Hettema *et al.*, 2001; Jacob *et al.*, 2010; Smoller *et al.*, 2009). In addition, nesting behavior is a highly polygenic trait as demonstrated by quantitative genetic analyses (Bult and Lynch, 2000; Lynch 1980), which is consistent with OCD in humans likely being influenced by many different genes. In twin studies of humans, concordance rates among monozygotic twins are generally significantly higher than concordance rates in dizygotic twins (Hettema *et al.*, 2001; Jacob *et al.*, 2010; Smoller *et al.*, 2009). Familial components in humans have also been identified, including increases in prevalence of OCD among first-degree relatives (Jenike, 2004; Pauls and Alsobrook, 1999; Stein, 2002). The genetic contributions to the compulsive-like nesting behavior support the nesting mouse model of OCD.

Studies on deer mice (Korff *et al.*, 2008, 2009) and rabbits (Hoffman and Morales, 2009) have also made significant progress towards the establishment of spontaneous and predictable animal models of OCD. Spontaneous stereotypic behaviors, including somersaulting, jumping, and pattern running, in deer mice were reduced significantly by chronic (3-week) fluoxetine treatments (10 and 20 mg/kg i.p.), but not by chronic desipramine treatments (10 and 20 mg/kg i.p.), while activity, quantified as total distance traveled, was not affected (Korff *et al.*, 2008). These findings of predictive validity are qualitatively similar to the findings in the BIG mice. In addition, the deer mice reduced the stereotypic behaviors significantly after subchronic (4-day) i.p. treatments with the 5-HT_{2A/C} agonist *meta*-chlorophenylpiperazine (mCCP) and D2 agonist quinpirole adding predictive validity, although effects on activity were not assessed (Korff *et al.* 2008). Construct validity for this animal model is suggested by the finding that deer mice with high levels of stereotypic behaviors have elevated cyclic adenosine monophosphate levels and reduced phosphodiesterase levels in the prefrontal cortex that are modified by chronic fluoxetine treatment (Korff *et al.*, 2009). Pregnant female rabbits build nests of straw a few

days before parturition, and after the nest is completed nest-building behavior is inhibited. The inhibition of this maternal nesting behavior depends on the completion of the nest as removal of straw prolongs the nesting behavior. A completed nest however is not a sufficient signal for the inhibition to occur if the female did not complete the nest herself, indicating that engaging in the behavior is important as well. These findings provide face validity of this rabbit model of OCD, with specific reference to “just right” perceptions (Hoffman and Morales, 2009).

In conclusion, the proposed nest-building animal model is novel in that it is a spontaneous non-induced behavioral model that shows face and predictive validity and could be of great benefit to the study of OCD and other compulsive spectrum disorders. Once established as an animal model, the compulsive-like nest-building mouse model may be used as a screening tool for drug and therapeutic treatments as well as for investigating neural mechanisms. Through the use of this mouse model to gain insights in the neural mechanisms controlling compulsive-like behaviors, the development of more effective treatments and a potential cure for compulsive spectrum disorders may be possible.

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Figure 1. Marble-burying behavior in BIG and SMALL male mice.

Marble-burying behavior was quantified by the number of marbles buried during a 20-min test period. Significant differences are shown as * $p < 0.05$, Tukey. All values are expressed as means $\pm$ SEM.

Figure 2. The effects of fluoxetine treatment on nest-building behavior in BIG male mice.

The data are expressed as a percent of the average of pre-drug weeks 3 and 4 for each drug and control group so that the groups could be directly compared. Significant differences from the control group are indicated * $p < 0.05$, Tukey. All values are expressed as means $\pm$ SEM.

Figure 3. The effects of fluoxetine treatment on marble-burying behavior in BIG male mice. Marble-burying behavior was quantified by the number of marbles buried during a 20-min test period. Significant differences from the control group are indicated * $p < 0.05$ -Tukey. All values are expressed as means $\pm$ SEM.

Figure 4. The effects of fluoxetine on locomotor behavior of BIG male mice.

Week '0' indicates the average of weeks 2-5 prior to drug treatment, all other weeks are normalized using this average. Mean ($\pm$ SEM) daily numbers of wheel revolutions per 5-min bin are shown.

Figure 5. The effects of clomipramine treatment on compulsive-like nest-building behavior of BIG male mice.

The data are expressed as a percent of the average of pre-drug weeks 2 and 3 for each drug and control group so that the groups could be directly compared. Significant differences from the control group are indicated * $p < 0.05$, Tukey. All values are expressed as means $\pm$ SEM. **A.** a 20 mg/kg/day oral dose of clomipramine for 8 weeks followed by a 80 mg/kg/day dose for 4 weeks. **B.** a 20 mg/kg/day oral dose of clomipramine for 8 weeks followed by a 40 mg/kg/day dose for 4 weeks.

Figure 6. The effects of desipramine and fluoxetine on nest-building behavior in BIG male mice. The data are expressed as a percent of the average of pre-drug weeks 2 and 3 for each drug and control group so that the groups could be directly compared. Significant differences from the control group are indicated * $p < 0.05$, Tukey. All values are expressed as means $\pm$ SEM.

Table 1

Open field behavior of SMALL and BIG mice.

	<i>SMALL nest-builders</i> (n = 10)	<i>BIG nest-builders</i> (n = 10)
Time (sec) to leave inner zone (latency)	48.8 ± 17.5**	5.0 ± 1.3
Time (sec) spent in inner zone	58.6 ± 19.0*	13.2 ± 2.8
Time (sec) spent in outer zone	126.8 ± 18.8**	147.2 ± 2.5
Number of rears in outer zone	2.6 ± 1.0**	9.3 ± 1.7
Time (sec) spent rearing	4.5 ± 1.9*	17.0 ± 2.7
Distance traveled (cm)	1163.7 ± 151.9*** ^a	2270.7 ± 180.5

Significance levels are indicated as

All values are expressed as means ± SEM.

^a n=8; two SMALL mice could not be traced by EthoVision for determination of distance traveled.*
p<0.05**
p<0.01***
p<0.0001.

Table 2

Elevated plus maze performance of BIG and SMALL mice and the impact of a previous behavioral test (open field behavior).

	<i>SMALL nest-builders</i>		<i>BIG nest-builders</i>	
	Experimentally experienced (n=10)	Experimentally naïve (n=5)	Experimentally experienced (n=10)	Experimentally naïve (n=5)
Total arm entries	15.8 ± 3.1	6.6 ± 1.9 [*]	22.8 ± 4.0	23.4 ± 2.7 ⁺⁺
Closed arm entries	12.1 ± 2.4	5.8 ± 1.7 [¥]	15.9 ± 2.6	14.8 ± 2.3 ⁺⁺
Open arm entries	3.7 ± 1.1	0.8 ± 0.9 [*]	6.9 ± 2.1	8.6 ± 3.1 ⁺⁺
Time in center (sec)	65.9 ± 12.3	41.6 ± 12.7	59.9 ± 9.7	80.8 ± 8.7 ⁺⁺
Time in closed arm (sec)	212.0 ± 13.7	247.5 ± 18.8	189.3 ± 17.6 [¥]	160.7 ± 27.7 ⁺⁺
Time in open arm (sec)	22.2 ± 7.3	10.7 ± 12.0	50.8 ± 10.9 ⁺	58.2 ± 19.6 ⁺⁺

Significance levels are indicated as

^{*}
p<0.05

[¥]
p=0.0576 for comparison of experimentally-experienced and experimentally-naïve SMALL male mice

⁺
p<0.05

⁺⁺
p<0.01 for comparison of experimentally-experienced SMALL and BIG male mice

^x
p<0.05 for comparison of experimentally-naïve SMALL and BIG male mice. All values are expressed as means ± SEM.