Selective breeding for dominant and submissive behavior in Sabra mice

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\section*{A B S T R A C T}

Background: The Dominant–Submitive Relationship (DSR) model used here was developed for mood stabilizing and antidepressant drug testing. Treatment of submissive animals with known antidepressants significantly reduced submissive behavior in a dose-dependent manner. We hypothesized that if submissive behavior in DSR is a valid model of depression, it should be possible to show a genetic predisposition for this trait, since clinical studies support a genetic component for depression.

Methods: To test this hypothesis, we applied selective breeding on outbred Sabra mice based on DSR paradigm.

Results: Here we have demonstrated that the frequency of DSR formation gradually increased across four generations of outbred Sabra mice, when animals inbred for the dominant trait were paired with those inbred for the submissive trait. Chronic imipramine administration (10 mg/kg) significantly reduced submissive behavior in the F2 generation consistent with the effect seen in unselected C57BL/6J mice.

Conclusions: We conclude that increased frequency of DSR formation suggest a genetic component of these two phenotypes, and strengthens the predictive and face validity of the DSR test. Selective breeding may aid in a better understanding of the genetic basis of dominant and submissive behavior, important elements in the etiology of affective disorders.

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1. Introduction

Dominant–Submitive Relationship (DSR)-based tests, the reduction of dominant behavior model (RDBM) and the reduction of submissive behavior model (RSBM), were developed to study the effects of chronic anti-manic or antidepressant drug treatments to facilitate the development of new medications for mania and depression (Malatynska et al., 2002; Malatynska and Knapp, 2005; Malatynska and Kostowski, 1984; Malatynska et al., 2007a, b; Malatynska et al., 2005; Pinhasov et al., 2005). In this test, drugs used in the clinic to treat mania reduced dominant behavior (Malatynska and Knapp, 2005; Malatynska and Kostowski, 1984), while drugs used to treat depression reduced animals' submissive behavior (Malatynska et al., 2002; Malatynska and Knapp, 2005; Malatynska et al., 2005; Pinhasov et al., 2005).

Depending on the selection criteria, from 25% to 40% of animals (rats or mice) form DSR (Malatynska et al., 2002; Malatynska and Kostowski, 1984; Malatynska et al., 2007b; Pinhasov et al., 2005). The rest of the animals do not form statistically distinguishable dominant–submitive relationships (for review see (Malatynska and Knapp, 2005)). Thus, DSR pair selection, which is the first and most important step in these paradigms, gives a consistent measure of the fraction of the population showing susceptibility to the expression of DSR behavior.
Developing lines of animals based on behavioral features is an important approach to study the biochemical and genetic basis of a trait of interest. Several lines of animals were developed to study the genetic and biochemical origins of anxiety and depression in rats (Malatynska and Kostowski, 1984; Pinhasov et al., 2005) and mice (Malatynska et al., 2007). The DSR paradigm was performed as described in rats (Malatynska and Kostowski, 1984; Pinhasov et al., 2005) and mice (Malatynska et al., 2007). The DSR paradigm was performed as described in rats (Malatynska and Kostowski, 1984; Pinhasov et al., 2005) and mice (Malatynska et al., 2007) with minor modifications. The apparatus (Fig. 1A) was constructed of red Plexiglas, chosen to reduce outside disturbances. It consists of two identical compartments (12 x 8.5 x 7 cm) joined by a tunnel (2.5 x 2.5 x 27 cm). A 0.5 cm diameter hole was cut in the bottom center of the tunnel. A self-refilling feeder (Fig. 1B) is connected to the tunnel, allowing a constant supply of sweetened milk (3% fat, 10% sugar). The tunnel had narrow slits cut on both sides of the feeder for easy gate insertion and removal (Fig. 1A). In this way, the paired mice had an equal time starting position at the beginning of the session.

DSR tests were carried out on five consecutive days per week. During each 16 h period preceding testing, the mice were deprived of food, but water was provided ad libitum. The animals had free access to food for two days between testing periods until the night before the next five-day testing period. The animals were housed in groups of five per home cage. The pairs of mice with relatively similar weight (average weight 24 ± 3 g) and from different home cages were tested daily. DSR testing of each pair was conducted for a single 5 min session on each day of the consecutive five-day testing period. During this 5 min session milk drinking scores were recorded manually by a human observer.

DSR testing was conducted over a two week period to select mice for breeding. The first week was used to adapt the mice to the testing conditions, while the selection of pairs showing a DSR was based upon scores measured during the second week. Dominant or submissive status for mice in a pair was defined on the basis of three criteria (Malatynska and Knapp, 2005; Malatynska et al., 2007b; Pinhasov et al., 2005). First, there had to be a significant difference between the average daily drinking scores of both animals in a pair. Second, the dominant animal’s drinking time had to be at least 40% greater than the submissive animal’s drinking time. Third, there must be no “turnaround” during second week of DSR testing, where the submissive animal’s drinking time is higher than its dominant partner on isolated occasions. The member of a pair that had a significantly lower drinking time (p < 0.05) in the selection week and fit the remaining selection criteria was defined as submissive and its counterpart was defined as dominant.

2. Materials and methods

2.1. Animals

Two-month-old outbred Sabra mice (Harlan Laboratory, Jerusalem, Israel) were subjected to DSR test-based selective breeding in-house. Sabra mice were originally developed at the Hebrew University, Jerusalem, Israel, and Harlan Laboratories, Ltd. has been maintaining this strain by contract with the Hebrew University since 1993. Animals were given standard laboratory chow and water available ad libitum. The colony room was maintained on a 12 h L:12 h D cycle (lights on 01:00–13:00 h). The experiments were conducted in compliance with the NIH/USDA guidelines, under the approved Institutional Animal Care and Use Committee (Protocol number IL-13-10-06).

2.2. Dominant–Submissive Relationship test

The DSR paradigm was performed as described in rats (Malatynska and Kostowski, 1984; Pinhasov et al., 2005) and mice (Malatynska et al., 2005), with minor modifications. The apparatus (Fig. 1A) was constructed of red Plexiglas, chosen to reduce outside disturbances. It consists of two identical chambers (12 x 8.5 x 7 cm) joined by a tunnel (2.5 x 2.5 x 27 cm). A 0.5 cm diameter hole was cut in the bottom center of the tunnel. A self-refilling feeder (Fig. 1B) is connected to the tunnel, allowing a constant supply of sweetened milk (3% fat, 10% sugar). The tunnel had narrow slits cut on both sides of the feeder for easy gate insertion and removal (Fig. 1A). In this way, the paired mice had an equal time starting position at the beginning of the session.

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2.3. Selective breeding

For breeding, a single dominant or submissive male was housed together with two or three female mice selected for the same trait. Females were removed and relocated into individual cages two weeks later. At 21–25 days from birth, offspring were weaned from the mother, weighed, ear clipped for identification, and housed in groups of five by sex throughout all experimental phases of the project.

Selective breeding experiments were started in August 2007. The founder dominant or submissive animals were designated generation P; offspring generations were designated F1, F2, F3, and F4. Males of the P generation that formed DSR during two week test period were bred with females with the same traits: submissive males with submissive females and dominant males with dominant females. In each subsequent generation, the number of animal pairs subjected to the DSR test was dependent on the number of offspring (Table 1). In consecutive (F1–F4) generations, offspring of the dominant mice were paired and subjected to the two-week DSR test with offspring of the submissive animals. Since a relatively small number of animals of P and F1 generations developed DSR, animals which met partially the defined criteria were also used for selective breeding. Thus, P and F1 animals with drinking time difference of less than 40% were
also used for selective breeding. This strategy was applied only for P and F1 animals. Inbreeding was minimized by breeding animals that were least related to one another.

Concurrently, we maintained a line of Sabra mice in which breeders were randomly assigned without any behavioral selections. This control group of animals was used to assess heritability of dominant/submissive traits.

### 2.4. Elevated plus-maze (EPM)

The elevated plus-maze was constructed with two open (10×45 cm) and two enclosed arms (10×45×40 cm) that extended from a common central platform (10×10 cm). The apparatus was made from black wood and was elevated on a wooden box to a height of 60 cm above floor level. Experiments were performed under dim illumination between 10:00 AM and 12:00 AM. One hour prior to the test the

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**Table 1**

Number of Sabra mice pairs forming Dominant–Submissive Relationships during four consecutive generations of selective breeding.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Generation</th>
<th>Number of tested animal pairs</th>
<th>Number of pairs that formed DSR</th>
<th>Percent (%) of pairs that formed DSR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>P</td>
<td>12</td>
<td>4</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>F1</td>
<td>10</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>F2</td>
<td>10</td>
<td>6</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>F3</td>
<td>20</td>
<td>13</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>F4</td>
<td>20</td>
<td>15</td>
<td>75</td>
</tr>
<tr>
<td>Females</td>
<td>P</td>
<td>8</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>F1</td>
<td>10</td>
<td>4</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>F2</td>
<td>10</td>
<td>6</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>F3</td>
<td>15</td>
<td>10</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>F4</td>
<td>20</td>
<td>16</td>
<td>80</td>
</tr>
</tbody>
</table>
animals were placed in the experimental room for habituation. Baseline testing was followed by one 5 min test for each mouse. The number of entries onto open and closed arms and the times spent in open and closed arms were manually scored. The maze was cleaned with 75% ethanol solution and rinsed with water after each test (Gobshtis et al., 2007).

2.5. Open field activity

Animals were placed in a transparent "open field" glass box (30×40 cm, divided into 20 squares of equal size). Recording of horizontal (the number of squares crossed) and vertical (the number of rears) activities have been done manually for 9 min (Gobshtis et al., 2007). The glass box was cleaned with water after each test.

2.6. Drugs and treatment

Submissive mice from the F2 generation were treated with imipramine hydrochloride (Sigma-Aldrich, Cat no. I0899) at 10 mg/kg, while dominant mice received injections of the vehicle alone (sterile water) for four consecutive weeks, starting at the beginning of the third week after selection and ending after the sixth week of the experiment. The solutions were administered by the intraperitoneal (i.p.) route.

To test the stability of the DSR reaction, dominant and submissive F4 generation animals were treated (i.p.) with the vehicle for three consecutive weeks starting at the beginning of the third week and ending after the fifth week of the DSR test.

2.7. Data analysis

Animals from parental and offspring generations were tested for DSR formation for two weeks. For each pair of mice, data were tested for statistical significance using the two-tail t-test. All pairs that met the criteria described above were selected. Two weeks' data for these selected dominant and submissive mice were plotted daily and were fitted using a non-linear regression analysis.

Dominance level values were calculated to measure the social relationship between paired subjects. Dominance level (DL) is calculated as the difference between the total daily feeding time of the dominant member of a pair and that of its submissive partner. Data were fitted by non-linear regression analysis to measure relationship onset time (ROT). ROT was estimated from the curve as the time to reach 50% of the maximum DL response.

Data from the imipramine and vehicle treatment study were averaged for each week for dominant and submissive mice and were fitted by non-linear regression analysis.

Non-linear regression analysis was done using GraphPad Prism software. Statistical significance of difference between dominant and submissive mice was evaluated using Two-Way ANOVA with Bonferroni-corrected post-hoc t-test analysis.

3. Results

In the parental (P) generation, 33% of Sabra males and 25% of the females formed DSRs (Table 1, Fig. 3A and F). The first generation of offspring (F1) produced 50% of pairs with DSR among males and 40% among females (Table 1, Fig. 3B and G). Progressively greater numbers of pairs developing DSR were observed in F2, F3, and F4 generations: 60%, 65%, and 75% among males and 60%, 66%, and 80% among females, respectively (Table 1, Fig. 2). There was significant difference in number of pair formations between generations (p<0.05). Bonferroni-corrected post-hoc analysis showed significant difference only in number of pairs formed between parental and F3 and F4 generations. There was no statistically significant difference between the number of DSR pairs formed by males and by females.

In the parental generation, the differences in time spent on the feeder by the paired mice was significantly different for males (p<0.01) and females (p<0.05) in the second week evaluation period. This difference was not significant in the first week acclimation period (Fig. 3A and F). For all filial generations, the time spent on the feeder by paired animals started to be significantly different during the first week acclimation period. The significant difference in time spent on feeders by dominant versus submissive males in the F1 generation begins on day four for both sexes (Fig. 3B and G; p<0.001) and in the F2 generation it appears for the first time on day five for males and on day four for females (Fig. 3C and H; p<0.0001). In the F3 generation this difference is noticeable as early as day one for females (Fig. 3I), but not until the fourth day for males (Fig. 3D; p<0.0001). Similar pattern of DSR formation onset was observed in the F4 generation. This is shown for males on Fig. 3E and for females on Fig. 3J.

For better comparison, the relation onset time (ROT) was calculated from non-linear regression analysis performed
using the dominance level of pairs from different generations (Fig. 4A and B). The ROT for F1 (3.1 ± 0.4 days), F2 (4.9 ± 0.6 days), F3 (3.6 ± 0.4 days), and F4 (3.6 ± 0.3 days) generations in males and for F1 (3.1 ± 0.3 days), F3 (3.8 ± 0.8 days), and F4 (1.7 ± 0.3 days) generations in females were significantly shorter than the ROT for parental animals (P1 males, 8.0 ± 0.6 days; P1 females 8.5 ± 0.4 days; Fig. 4C). In the F2 females, the ROT of 8.0 ± 0.7 days was not significantly different from the parental females (Fig. 4C). The ROTs for males and females were not significantly different with the exception of the F2 groups.

The effect of imipramine on submissive behavior was examined on F2 Sabra males. Daily imipramine injections (i.p.) at a dose of 10 mg/kg for four weeks gradually reduced their submissiveness (Fig. 5A). In this experiment, a significant difference in drinking time between groups of dominant and submissive animals was already present after the first week of testing (p < 0.001) and increased during the second week (p < 0.0001, Fig. 5A). Submissive animals were injected daily with imipramine, and their dominant counterparts were injected with sterile water from the start of the third week of the study. A reduction of submissive behavior was observed after the second week of imipramine treatment, and was eliminated after the third and fourth weeks of treatment (Fig. 5A).

**Fig. 4.** Dominance levels in pairs of male (A) or female (B) Sabra mice that formed Dominant–Submissive Relationship (DSR) across the P, F1–F4 generations. The onset of DSR of the P animals was compared to the filial generations (C). Data points marked (*) are significant at p < 0.05, (**) at p < 0.01, and (****) at p < 0.001. Number of pairs in each generation was as follow for males: P (n = 4), F1 (n = 5), F2 (n = 5), F3 (n = 13) and F4 (n = 15); for females: P (n = 4), F1 (n = 5), F2 (n = 5), F3 (n = 13) and F4 (n = 16).

**Fig. 5.** Effect of chronic 10 mg/kg i.p. imipramine (IMI) administration on behavior in submissive Sabra mice of the F2 generation (A). Effect of chronic i.p. injection of vehicle (sterile water) on dominant–submissive behavior (B). Changes in dominance level of imipramine-treated compared to vehicle-treated animals (C). Data points significantly different between dominant (Dom, n = 5) and submissive (Sub, n = 5) groups of animals are marked (*) at p < 0.05, (**) at p < 0.01, and (****) at p < 0.001.
The stability of the Dominant–Submissive Relationships was tested on males of the F4 generation. After selection, both dominant and submissive mice were treated (i.p.) with sterile water for three consecutive weeks. Chronic vehicle administration did not change previously established DSRs among these animals (Fig. 5B). A comparison of dominance levels between animals treated with imipramine and those who received vehicle is depicted on Fig. 5C.

Sabra male that were subjected to selective breeding were evaluated with the Elevated plus-maze (EPM) and Open Field tests. No significant differences in behavior between dominant, submissive and control (randomly bred) groups were found in the EPM test (Table 2). The Open Field test did not reveal any changes in the horizontal activity between experimental groups (Fig. 6A). However, a significant difference in the vertical activity (rearing) was found between F4 submissive and control Sabra males (Fig. 6B).

4. Discussion

This work’s major findings are twofold: that breeding Sabra mice for dominant and submissive traits resulted in progressively larger numbers of pairs in each consecutive generation of animals meeting the DSR criteria, and that statistically significant DSRs were formed more rapidly in filial than in the parental generations. These findings suggest that Dominant–Submissive Relationships defined in the DSR test have an inheritable genetic component. This finding supports the face validity of the RSBM described here as a disease model of depression since clinical studies suggest that major depression has a genetic component. This conclusion is strengthened by the use of outbred animals that are more genetically diverse than the inbred strains used in previous studies. Like the other mouse strains studied, the outbred Sabra mice form discrete numbers of DSR pairs, and the submissive members of these pairs respond to imipramine treatment with reduced submissive behavior. While encouraging, this is a limited result and further studies need to be done to support the use of these mice in this behavioral model. Previously we have shown that mice can form DSR using the inbred C57BL/6J strain (Malatynska et al., 2005). We show here that Sabra animals are also capable of developing stable Dominant–Submissive Relationships (Fig. 3A and F).

Several researchers have used increased frequency of a trait in populations subjected to selective breeding as a measure of the genetic component of the trait under investigation

Table 2

Behavior data of four generations (P, F1,3,4) of Sabra dominant (Dom), submissive (Sub) and control male mice in the EPM test.

<table>
<thead>
<tr>
<th>Generation</th>
<th>Dom (n = 4)</th>
<th>Sub (n = 4)</th>
<th>Cont (n = 8)</th>
<th>F1</th>
<th>Dom (n = 5)</th>
<th>Sub (n = 5)</th>
<th>Cont (n = 10)</th>
<th>F3</th>
<th>Dom (n = 10)</th>
<th>Sub (n = 10)</th>
<th>Cont (n = 12)</th>
<th>F4</th>
<th>Dom (n = 16)</th>
<th>Sub (n = 16)</th>
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<tr>
<td></td>
<td>Mean</td>
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<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
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<td>Mean</td>
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<tr>
<td>P</td>
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<td>2.68</td>
<td>11.38</td>
<td>2.28</td>
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<td></td>
<td>21.75</td>
<td>1.11</td>
<td>17.75</td>
<td>0.85</td>
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<td>1.40</td>
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<td>106.00</td>
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<td>6.29</td>
<td>112.90</td>
<td>5.83</td>
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<td>136.6</td>
<td>10.96</td>
<td>106.00</td>
<td>6.70</td>
<td>124.30</td>
</tr>
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</table>

The values are means ± SEM with the number of animals for each generation.

OE – open arms entries; CE – closed arms entries; TE – total entries; OT – open arms duration (s); CT – closed arms duration (s).

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(Hitzemann et al., 2008; Pulido et al., 1996; Verle et al., 2000). For example, genetic involvement in catalepsy was shown by the increasing percent of animals with catalepsy across generations of animals selected for this trait (Kondaurova et al., 2006). Breeding submissive and dominant animals separately strengthened the presence of these two behavioral traits in the population, as reflected in the greater number of pairs forming DSR, and by the shorter time needed to develop DSR between paired animals in later generations. There was a progressive increase in the number of pairs forming DSR across generations (parental and four generations of offspring) as shown in Fig. 2A. Statistically significant differences in post hoc analysis were achieved between parental and F3 and F4 generations. Two-Way ANOVA showed no significant difference between number of pairs formed by males and females across generations. This finding is consistent with our previous results conducted on Sprague Dawley rats showing no difference between sexes in number of pairs developing DSR (Malatynska et al., 2002).

The second measure of the genetic component of the traits under investigation was latency of DSR formation. In randomly bred Sabra as well as C57BL/6J mice and Sprague Dawley rats, significant DSR developed in the second week of interaction (Fig. 3A and F (Malatynska et al., 2002; Malatynska and Knapp, 2005; Malatynska et al., 2007b)). To compare the DSR latency between generations in our work, we defined a relationship onset time (ROT) value as the time necessary to reach 50% of the maximal dominance level of the DSR pairs of a given generation. Maximal dominance level was derived from non-linear regression analysis (Fig. 4A and B). Generally, the latency of DSR development was significantly shorter for selectively bred male and female Sabra mice than for randomly bred Sabra mice (Fig. 4C). This may indicate a genetic component of dominant and submissive traits. Although ROT values remained constant across offspring generations, some non-linearity occurred in the F2 generation (Fig. 4C). This ceiling effect, which appeared exclusively in generation F1 may be attributed to several factors, including relatively small sample size, analysis of two traits simultaneously, and the complexity of inheritance.

It is well established that valid animal models of diseases such as depression or mania should be characterized by three measures: predictive, face, and construct validity (Willner, 1984, 1995). Genetic control would support the face validity of our work, which is based on the presence of neurochemical changes observed in the model and during the illness and genetic control is expressed by neurochemical features observed in psychiatric disorders. There is common agreement that the spectrum of bipolar disorders is under the control of several genes (Carter, 2007; Elashoff et al., 2007). There are several reports related to genetic aspects of dominance and subordination traits (Chapais and Shulman, 1980; Jarrell et al., 2008; Jozil’kova and Plegr, 2006; Kaesermann and Baettig, 1984, 1995). Genetic control would support the face validity of our work, which is based on the presence of neurochemical changes observed in the model and during the illness and genetic control is expressed by neurochemical features observed in psychiatric disorders. There is common agreement that the spectrum of bipolar disorders is under the control of several genes (Carter, 2007; Elashoff et al., 2007). There are several reports related to genetic aspects of dominance and subordination traits (Chapais and Shulman, 1980; Jarrell et al., 2008; Jozil’kova and Plegr, 2006; Kaesermann and Baettig, 1984, 1995; McCobb et al., 2003; Vera Cruz and Brown, 2007). However, the detailed nature of the genetic control of dominant and submissive traits remains to be elucidated. In this work, we produced data supporting that genetic mechanisms hold at least partial control over dominance and submissiveness in Sabra mice. Our findings are consistent with the study by Masur and Benedito (1974) which found that the frequency of dominant or submissive behavior increases among rats bred for these traits. The graded increase in pair forma-

It has been previously shown that submissive behavior of mice and rats measured in pair food competition tests was sensitive to antidepressants desipramine, imipramine, amoxapine, and fluoxetine (Malatynska et al., 2002; Malatynska et al., 2005; Pinhasov et al., 2005). In our study, we have shown that submissiveness of male Sabra mice is reduced by treatment with antidepressant imipramine (10 mg/kg) for three weeks and remains significantly reduced after a fourth week of treatment, the longest time studied (Fig. 5A). At the same time, chronic treatment of F4 animals with vehicle (sterile water) for three consecutive weeks did not affect animals’ dominant–submissive behavior (Fig. 5B). This is similar to the stability of controls in rat DSR (Malatynska et al., 2007b; Pinhasov et al., 2005). Analyses of animals’ behavior in the EPM and Open Field tests did not reveal any significant differences between dominant, submissive and control species (Table 2, Fig. 6A). The only significant difference between F4 submissive and control males has been observed in vertical activity (Fig. 6B). This phenomenon should be further evaluated. The results of our experiments strengthen the potential of submissive Sabra mice to serve as a model of depression. However, a full characterization of its predictive validity should be conducted. In summary, we have demonstrated genetic control of dominant and submissive behavioral traits as expressed in fixed-pair competition tests in Sabra mice. These results strengthen the face validity of DSR based models for depressive and manic disorders. Use of these selectively bred animals with prominent dominant and submissive features in drug testing could reduce the number of animals entering experiments, while increasing throughput for testing putative antidepressant or anxiolytic drugs. Clearer genotypes can aid in better understanding of submissiveness and dominance traits, which are important elements in the etiology of depressive and manic disorders, respectively.

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No conflict declared.

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References


