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ARTICLE

Inherited behaviors, BDNF expression and response to treatment in a novel multifactorial rat model for depression

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Abstract

Major depressive disorder (MDD) is a common and devastating mental illness behaviorally characterized by various symptoms, including reduced motivation, anhedonia and psychomotor retardation. Although the etiology of MDD is still obscure, a genetic predisposition appears to play an important role. Here we used, for the first time, a multifactorial selective breeding procedure to generate a distinct 'depressed' rat line (DRL); our selection was based upon mobility in the forced swim test, sucrose preference and home-cage locomotion, three widely used tests associated with core characteristics of MDD. Other behavioral effects of the selection process, as well as changes in brain-derived neurotrophic factor (BDNF) and the response to three antidepressant treatments, were also examined. We show that decreased mobility in the forced swim test and decreased sucrose preference (two directly selected traits), as well as decreased exploration in the open field test (an indirectly selected trait), are hereditary components in DRL rats. In addition, lower BDNF levels are observed in the dorsal hippocampus of DRL rats, complying with the neurotrophic hypothesis of depression. Finally, electroconvulsive shocks (ECS) but not pharmacological treatment normalizes both the depressive-like behavioral impairments and the BDNF-related molecular alterations in DRL rats, highlighting the need for robust treatment when the disease is inherited and not necessarily triggered by salient chronic stress. We therefore provide a novel multifactorial genetic rat model for depression-related behaviors. The model can be used to further study the etiology of the disease and suggest molecular correlates and possible treatments for the disease.

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Introduction

Although 50% of the risk for major depressive disorder (MDD) is today considered genetic (Sullivan et al., 2000), identification of specific MDD-related genes has proved challenging. This is largely attributable to the multifactorial nature of the disease (American Psychiatric Association, 2000; Verhagen et al., 2008; Martin-Soelch, 2009) and methodological differences between studies (Lopez-Leon et al., 2008). One approach that is beneficial for identifying inheritable components of complex behavioral phenotypes is artificial selection in animal models, which allows controlled breeding along with replicable behavioral and molecular measurements (Finn et al., 2003; El Yacoubi and Vaugeois, 2007; Mackay, 2009). Thus, although the genes and neurophysiological mechanisms underlying such complex behavioral phenotypes may differ between humans and other mammals, animal models can help discover new therapies for psychiatric disorders, as well as provide mechanistic insights (Vollmayr et al., 2007).

Several studies previously attempted to establish MDD-like genetic animal lines through artificial selection (Overstreet, 2012; Wegener et al., 2012). For instance, early studies by Overstreet and colleagues selected rodents that generally demonstrated extreme responses to an acetylcholinesterase inhibitor (Overstreet et al., 1994) or to a 5-HT_{1A} agonist (Overstreet et al., 1996), and later studies attempted to generate an MDD-like animal model possessing somewhat higher face validity. This was accomplished by establishing a selection process based upon more behaviorally relevant tests: the tail suspension test (El Yacoubi et al., 2003), the forced swimming test (FST) (Weiss et al., 1998) or the elevated plus maze (Landgraf and Wigger, 2002). The limitation of these models is that the selection process was based upon single behavioral assays (whereas MDD is fundamentally diagnosed by a combination of several symptoms), and second that some of the behavioral assays used for selection are also associated

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with non-MDD disorders (American Psychiatric Association, 2000; Fava and Kendler, 2000). A multifactorial artificial selection process based on established depressive-like behaviors may therefore result in a more relevant model for MDD; however, such endeavor has not been previously accomplished.

Here we artificially selected wild-type (WT) naïve Sprague–Dawley rats for 10 consecutive generations of inbreeding. The selection process was based only on the rats' natural behavioral traits, as assessed through their performance in three independent behavioral measurements closely associated with the core symptoms of MDD: (1) the FST, which is considered a measure of motivation and is often used as a high throughput tool for screening potential antidepressant medications (Porsolt et al., 1977); (2) sucrose preference, which is considered a measure of anhedonia (Overstreet, 2012); and (3) home-cage locomotion, which is considered a measure for psychomotor retardation (Dedic et al., 2011). Our selection process was bi-directional: rats with lowest performance in these tests were inbred among themselves, and rats with the highest performance in these tests were also inbred among themselves. This resulted in two behaviorally distinct rat lines: a 'depressed' rat line (DRL, which showed the lowest performance) and a 'motivated' rat line (MRL, which showed the highest performance). In addition, as converging lines of evidence point to a critical role of brain-derived neurotrophic factor (BDNF) in MDD and MDD-like behaviors (Duman, 2002; Elfving et al., 2010; Taliaz et al., 2010), we also characterized BDNF expression in specific sites of the brain's reward system to directly test whether inherited depressive-like behavioral traits are accompanied by inherited BDNF alterations. Finally, we evaluated the effects of three different antidepressant treatments that are successfully used in animal models (Grant and Weiss, 2001; Will et al., 2003; Gersner et al., 2010): the tricyclic drug desipramine, the selective serotonin reuptake inhibitor (SSRI) fluoxetine, and electroconvulsive shock (ECS) therapy, which is a highly effective non-pharmacological treatment for MDD.

Methods

Animals

All experiments were conducted in accordance with National Institutes of Health (NIH) guidelines for the care and use of laboratory animals. Sprague–Dawley rats were maintained under a 12 h light/12 h dark cycle with food and water supplied *ad libitum*. Behavioral testing began at 60 d of age, at which time rats were singly housed in Perspex home cages of 18×26×40 cm. Following the behavioral procedures, one male and one female were selected and housed together for breeding.

Selective breeding

The parental generation, F0, consisted of 16 male and 16 female WT naïve rats. Without any prior intervention, each of these F0 rats underwent a thorough multifactorial behavioral assessment through three depression-related tasks (see below). Assuming a naturally occurring phenotypic population variability in performance in these tasks, a 'selection index' was then calculated for each individual rat that evaluates its overall performance in the three tasks combined, relative to the performance of other rats in the F0 generation (see below); thus, a low 'selection index' represents low performance (i.e. 'depressed'-like behavior) and a high 'selection index' represents high performance (i.e. 'motivated'-like behavior) in the these three tests combined, relative to other rats in the population. Then, the two males and two females that showed the lowest selection index were bred together to produce the first generation (S1) of the 'depressed' rat line (DRL), and the two males and two females that showed the highest selection index were bred together to produce S1 of the 'motivated' rat line (MRL). This was then repeated separately in each group and in each generation, such that the 2–3 DRL pairs that showed the lowest selection index were bred together to continue the DRL line, whereas the 2–3 MRL pairs that showed the highest selection index were bred together to continue the MRL line. To verify that DRL and MRL characteristics are determined genetically rather than *postnatally* (e.g. maternal behavior), half of the MRL and DRL pups were cross-fostered and raised by WT surrogates at the sixth generation of breeding. Cross-fostering did not influence the behavior of rats, pointing to genetic source of the observed phenotypes.

Behavioral testing

The order of the behavioral tests is depicted in Fig. 1. Rats were grouped in same-sex quartets in a single cage until 60 d of age, after which they were housed with one rat per cage and underwent all behavioral tests within a 4-wk period. To avoid irrelevant behavioral biases owing to female menstrual cycle, further behavioral analyses were conducted on males only. In addition, males from the S5–S8 generations which were not chosen as fathers for the next generations were again subjected to the same behavioral tests after receiving antidepressant treatments. Thus, for these animals, each behavioral test was repeated twice within a two-month period. Indirect responses to selection were tested at the ninth generation of selective breeding by measuring performance in elevated plus maze (EPM) and open field test (OFT). In addition, the body mass at 30, 60 and 90 d of age was measured in rats from ninth and tenth generations.

Behavioral assays

Home-cage locomotion (HCL). At 60 d of age, the baseline HCL was determined for each animal for five

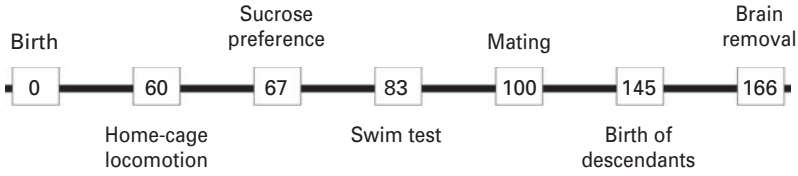

Fig. 1. Experimental timeline for a single generation of selective breeding. Age is presented in days post-partum. See Methods for further details.

successive days. A computerized system (Inframot©, TSE, Germany) sampled locomotion in 1-min bins for 24 h/d. For each 24-h period, night locomotion score was then summed for each cage and the average score of the five-night period is reported here.

Sucrose preference (SP). At 67 d of age, two drinking spouts were positioned side by side at the rear part of the home cage. Both drinking bottles were filled with filtered tap water in the first 2 d of the experiment, and then the water on the left bottle was replaced by a sucrose solution (0.2% in filtered tap water). Fluid consumption was recorded in 24-h bins for four successive days by weighing the bottles every day between 12:00 and 14:00 hours. Then, tap water was again filled in both bottles for 1 d, after which a sucrose solution was filled on the right bottle and tap water was filled on the left, and fluid consumption was tested for another 4 d. The sucrose preference score was calculated as the percentage of sucrose solution consumption of the total liquid consumption during the days in which the sucrose solution was provided.

Forced swim test (FST). At 83 d of age, a modified FST was conducted in a custom-built cylindrical water tank (40 cm high, 18 cm in diameter, water maintained at 26 °C). The water level was such that the rat could not touch the bottom. Rats were placed in the water for 10 min and their behavior (swimming, immobility or diving events) was noted and recorded with a high-definition digital camera. The recordings were then analyzed with a custom-built algorithm as described previously (Gersner et al., 2009) to produce the mobility score. All tests were performed between 10:00 and 18:00 hours.

Open field test (OFT). Rats from the ninth generation of selective breeding were placed in a 40×40 cm exploration box (ActiMot System Activity Chamber, TSE, Germany) in which the distance traveled, the number of rearings and the number of center visits were recorded automatically during a 10 min period. A 13.3 cm^2 area in the middle of the 40 cm^2 box was defined as the central square. Tests were performed between 10:00 and 18:00 hours.

Elevated plus-maze. A four-arm maze in the shape of a +sign was custom-built and raised 60 cm above the ground. The maze consisted of two open arms (50×10 cm, with a 0.5 cm rim) and two closed arms (50×10×32 cm), arranged such that arms of the same type were opposite of each other and connected by a central area (10×10 cm). Rats from the ninth generation were placed in the central area (head position counterbalanced among rats) and observed for 5 min under dim red light. The time spent in the open and closed arms was recorded by an experienced observer.

Body mass measurement. Rats from the ninth and tenth generations were weighed at 30, 60 and 90 d of age. The data were compared to supplier-provided weight values of Sprague–Dawley WT rats grown in similar conditions with the same diet.

Selection index. A score between 0–1 was assigned for each rat in each behavioral assay (FST, SP and HCL) according to the following procedure:

S_i is first defined as the raw score of the ith animal in n animals and the minimal and maximal raw scores within the same generation are denoted by Min() and Max(), respectively. A normalized score NS_i is then defined for the animal in each behavioral measurement to produce FST_i, SP_i and HCL_i, as the result of the following equation:

$$NS_i = \frac{S_i - \mathrm{Min}(S_1 \ldots S_n)}{\mathrm{Max}(S_1 \ldots S_n) - \mathrm{Min}(S_1 \ldots S_n)}$$

The selection index (SI) is then calculated for each animal according to the equation:

$$\mathrm{SI}_i = \frac{\mathrm{SP}_i + \mathrm{FST}_i + \mathrm{HCL}_i}{3}$$

For the parents' generation (F0), the SI was defined for each rat in the WT population. In further generations, the SI was calculated separately for the DRL and the MRL groups. Owing to lack of behavioral separation between DRL and MRL in the HCL score (see Fig. 2), the SI in generations S8–S10 was calculated based on the FST and SP scores only.

Antidepressant treatments

After the regular battery of behavioral tests, several DRL males that were not selected as fathers for the next generation received chronic daily antidepressant

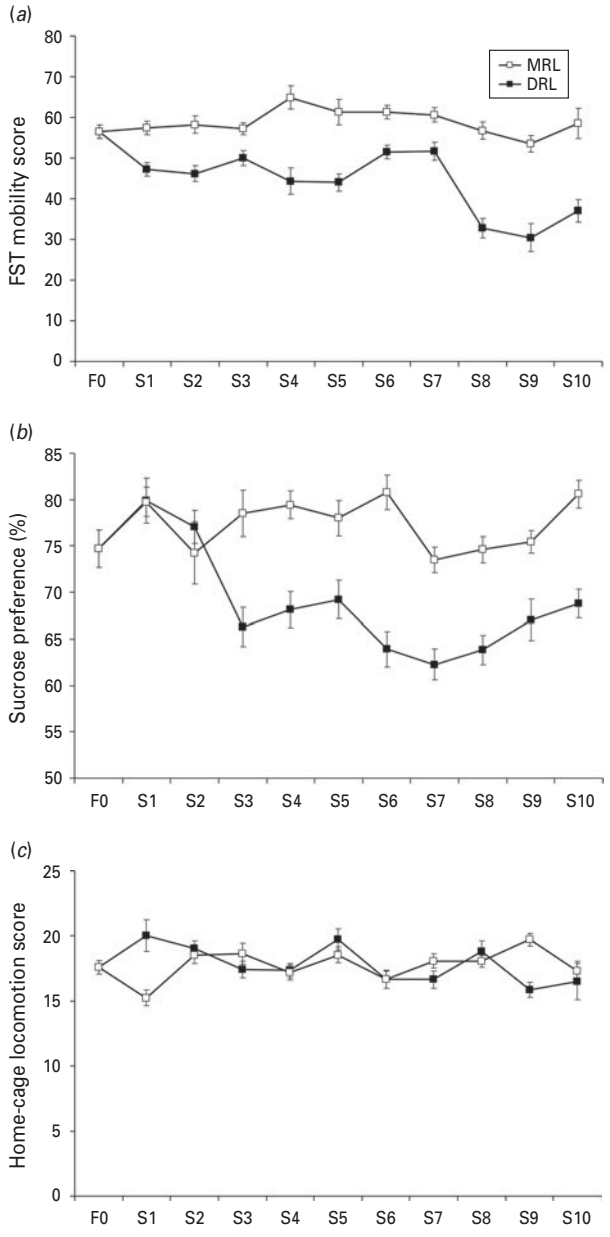

Fig. 2. Direct effects of selective breeding. Rats were selectively bred from wild-type (WT) rats (F0) for 10 consecutive generations (S1–S10) based on mobility in the forced swimming test (FST) (*a*), sucrose preference (*b*) and home-cage locomotion (*c*). In each generation, rats with the lowest (DRL, depressed rat line) or highest (MRL, motivated rat line) performance were chosen as parents for the next generation. Data points represent mean±S.E.M. See text for details.

treatment for 10 d. Then, they were again subjected to the same battery of behavioral tests and their behavior was scored. The antidepressant treatments used in this study were desipramine (a triciclic medication shown to be effective in remedy of depressive-like behavior in rats (Will et al., 2003)) and electroconvulsive shocks (ECS), which are considered the most effective antidepressant treatment available today (Pagnin et al., 2004).

Desipramine. A group of DRL animals (from generations S5–S6) received a daily dose of desipramine (15 mg/kg, i.p.) or saline (sham group) for 15 consecutive days.

Fluoxetine. A group of DRL animals received a daily dose of fluoxetine (10 mg/kg, i.p.) or saline (sham group) for 3 wk.

ECS. A group of DRL animals (from generations S7–S8) received a train of electroconvulsive stimulations (100 V, 50 Hz, 1.5 s, applied via ear-clip electrodes using Siemens Konvulsator 2077 S) for 10 consecutive days as was described previously (Gersner et al., 2010). Stimulation parameters were set to achieve a tonic seizure lasting at least 10 s under mild anesthesia (ketamine HCl 85 mg/kg and promace 0.85 mg/kg) according to Institutional Animal Care and Use Committee requirements. Sham (control) animals were treated similarly, without applying stimulation.

BDNF ELISA

Tissue punches. Following the battery of behavioral tests (see Fig. 1), animals from generations S5–S8 were sacrificed and their brains extracted, frozen in isopropanol and stored at −80 °C. Coronal sections were sliced in a cryostat at −20 °C to reach the appropriate region, and then bilateral tissue punches were extracted from the dorsal hippocampus (from −2.3 to −4.3 mm relative to bregma), ventral hippocampus (from −4.3 to −5.8 mm relative to bregma) and striatum (from +2.2 to +0.7 mm relative to bregma), using a manual cutter as described previously (Gersner et al., 2010).

Protein extraction. Protein extraction was performed as previously described (Baker-Herman et al., 2004). Brain tissue samples were weighed and homogenized in cold extraction buffer (Tris-buffered saline, pH 8.0, with 1% NP-40, 10% glycerol, 5 mM sodium metavanadate, 10 mM PMSF, 100 μg/ml aprotinin and 10 μg/ml leupeptin). Homogenates were acidified with 0.1 M HCl (pH ~3.0), incubated at room temperature for 15 min and neutralized with 0.1 M NaOH (pH ~7.6). They were then centrifuged at 7000 *g* for 10 min and supernatants were assayed using a standard sandwich enzyme-linked immunosorbent assays (ELISA) procedure.

ELISA protocol. Sandwich ELISA were carried out at room temperature using monoclonal mouse anti-human BDNF capture antibody (R&D systems, USA), 2.5 μg/ml in phosphate buffered saline (PBS). The capture antibody was incubated overnight in 96-well flat-bottomed polystyrene plates. After incubation, the wells were washed three times with a washing buffer (0.05% Tween 20 in PBS, pH 7.2–7.4). Then, 300 μl of a blocking buffer (1% bovine serum albumin (BSA), 5% sucrose in PBS with 0.05% NaN_3) were added to each well and the wells

were incubated for 1 h at room temperature. After three additional washes, brain homogenized samples (100 μl per well) were added in duplicates. Positive (BDNF) and negative (reagent diluent: 1% BSA in PBS pH 7.2–7.4, 0.2 μM filtered) controls were included. After 2 h of incubation (at room temperature) and washing, mouse anti-human BDNF (100 μl per well) biotinilated detection antibody (R&D systems, USA) diluted at 2.5 μg/ml in reagent riluent was added and the plates were incubated again for 2 h. After the wells were washed three times, streptavidin conjugated to horseradish peroxidase (R&D systems, USA) diluted 1:200 in reagent diluent was added (100 μl/well) and the plates were incubated in darkness for 20 min. After the wells were washed again three times, a substrate solution (1:1 mixture of color reagent H_2O_2, and color reagent Tetramethylbenzidine, Chemicon International, USA) was added at 100 μl/well. The color then developed for 20 min in darkness and the reaction was stopped with 50 μl 2 N H_2SO_4. The plates were read at 450 nM using a microplate reader (EL_x808, Bio Tek, USA).

Data analysis

Cumulative selective differential. To calculate the selective pressure of each test, behavioral scores were standardized (i.e. z-scores) as described elsewhere (Huynh et al., 2011). Namely, z-scores were produced by subtracting the mean score of WT rats (generation F0) from each observation and dividing the result by the S.D. of the WT rats. Next, for each strain in each generation, we calculated the selective differential between the mean behavioral z-score of rats chosen as parents for the next generation and the mean z-score of the entire generation. The cumulative selective differential in a specific generation was defined as the sum of selective differentials up to this generation, which thus reflects the selective pressure.

Statistics. Results are presented as means ± S.E.M throughout. One-way analysis of variance (ANOVA) was used to analyze behavioral differences, and significant main effects were followed by contrast analysis to allow weighed and simultaneous comparisons between all strains. For the OFT and EPM results, and for the behavioral results of DRL rats after antidepressant treatments, significant main effects were followed by Fisher's least significant difference *post-hoc* tests. Changes in body mass were analyzed by repeated-measures ANOVA with strain as the between-subjects factor and age as the within-subjects factor, and significant effects were followed by a two-tailed *t*-test for independent samples of strains to compare strains at different ages. For rats receiving antidepressant treatments, and owing to lack of differences between the antidepressant control groups (saline for desipramine and fluoxetine; sham treatment for ECS), the control conditions were combined into a single group. Data for the BDNF protein levels were analyzed separately for the effect of strain and for the effect of antidepressant treatment with one-way ANOVA. Significant main effects were followed by Fisher's least significant difference *post-hoc* tests. All performed tests were two-tailed, and *p*-values lower than 0.05 were considered statistically significant. Statistical analyses were performed using the Statistica 8.0 software (StaSoft Inc., USA).

Results

Direct behavioral responses to multifactorial selective breeding

Rats were selected based on their performance in three behavioral tests: the forced swimming test (FST), the sucrose preference (SP) test and the home-cage locomotion (HCL) test. Each test initially received an equal weight in the generation of an individual global performance index (see Methods). Male and female rats with the lowest ('depressed') or highest ('motivated') performance indices were mated together for 10 consecutive generations to ultimately result in two behaviorally distinct lines, termed 'depressed' rat line (DRL) and 'motivated' rat line (MRL). A behavioral trait was considered 'inherited' when the performance of the offspring was statistically significant from that of its parents in the direction of selective pressure.

Overall, we found significant inheritance of decreased but not increased performance in the FST and SP tests but not in the HCL test (Fig. 2). One-way ANOVA performed on the FST data over 10 generations of DRL, MRL and WT (F0) rats revealed a significant main effect ($F(20, 476)=14.183$, $p<0.0001$) and a contrast *t*-test revealed that the behavior of DRL ($p<0.0001$), but not of MRL, differed from that of WT rats across all 10 selected generations (Fig. 2*a*). Notably, the average reduction in FST mobility scores in DRL compared with the WT rats increased from 14.14% ($p<0.0001$) across generations S1–S7 to 41.81% ($p<0.0001$) across generations S8–S10. A significant behavioral separation between DRL and MRL began as early as generation S1 ($p<0.0001$) and persisted across all selected generations (Fig. 2*a*).

One-way ANOVA performed on the SP data revealed a significant effect of the group ($F(20, 536)=9.7$, $p<0.0001$) and a contrast *t*-test revealed that the behavior of DRL ($p=0.001$), but not of MRL, differed from that of WT rats (Fig. 2*b*). A significant behavioral separation between DRL and MRL began from generation S3 ($p<0.0001$) and persisted across further generations, with the average reduction in SP of DRL relative to WT rats was 11.9% ($p<0.001$) across generations S3–S10. The increase in SP in the MRL rats was not significant (Fig. 2*b*).

No significant response to selection was observed for the HCL test (Fig. 2*c*) and no significant differences were observed in this measurement between DRL, MRL and WT rats across generations. Owing to this lack of

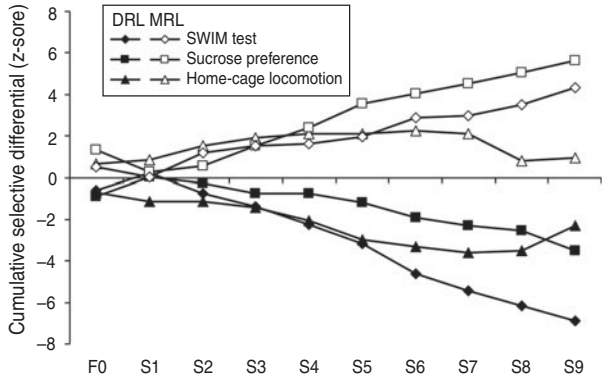

Fig. 3. Selective pressure increases with time in all behavioral measurements. For each of the three behavioral tests, the cumulative selective differential is presented as the z-score of 'depressed' and 'motivated' rat lines (DRL and MRL, respectively) in terms of the wild-type (WT) rats (zero line). Note the late decrease in the cumulative selective differential for home-cage locomotion owing to lack of selective breeding for this trait in generations S8–S9.

behavioral separation, and in order to increase the power of selective breeding, selection from generation S8 onwards was based on FST and SP scores only.

To estimate the magnitude of selective pressure we calculated the cumulative selective differential for the three behavioral tests (Fig. 3). This indicated that selective pressure was exerted on both DRL and MRL rats, but not equally for the three behavioral measurements. At the ninth generation of selective breeding, the cumulative selective differential for the DRL was −6.87 S.D. of WT values for the FST, but only −3.52 S.D. for SP and −2.29 S.D. for HCL. In the MRL, the cumulative selective differential was 4.35, 5.63 and 0.95 for the three tests, correspondingly. As expected, as rats from generation S8 onwards were selected based only on their FST and SP scores, the cumulative selective differential decreased for HCL (but not for FST or SP) in generations S8 and S9 (Fig. 3).

Indirect responses to multifactorial selective breeding

We next quantified indirect effects of the selection process in late (9th–10th) generations (Fig. 4). These effects, which are associated with MDD-like behavior in animal models but were not directly used for the selection process in the current study, include novelty-induced behaviors in the OFT anxiety assessment in the EPM and body mass measurements at days 30, 60 and 90 post-partum.

A significant effect for strain was found in all measured parameters in the OFT, namely in the total distance traveled in the novel arena ($F(2,47)=5.14$, $p=0.0096$), the number of rearings ($F(2,47)=9.05$, $p=0.0005$) and the number of center visits ($F(2,47)=7.49$, $p=0.0015$) (Fig. 4*a*). A *post-hoc* analysis revealed that the total distance and number of center visits were significantly decreased in DRL ($p=0.049$ and $p=0.041$, respectively), and that the number of rearings and center visits were significantly increased in MRL ($p=0.0017$ and $p=0.035$, respectively), compared with WT rats (Fig. 4*a*). In addition, DRL rats displayed significantly lower exploration of the novel environment compared with MRL in all three parameters (total distance: $p=0.0025$, number of rearings: $p=0.0004$, number of center visits: $p=0.0004$). Anxiety, as indexed by the EPM, was altered in MRL but not in DRL rats (Fig. 4*b*) and a significant effect was found for strain ($F(2,21)=5$, $p=0.0167$). *Post-hoc* comparisons revealed that the number of entrances to the open arms was similar in DRL and WT rats but was significantly elevated in MRL rats ($p=0.0187$). Finally, repeated-measures ANOVA on body mass (Fig. 4*c*) with strain as a between-subjects factor and age (30, 60 and 90 d post-partum) as a within-subjects factor revealed a significant main effect of strain ($F(2,180)=19.21$, $p<0.0001$) and a significant interaction between the two factors ($F(4,180)=45.2$, $p<0.0001$), indicating that the rate of weight gain is different between the strains. *Post-hoc* analyses revealed no difference between DRL and WT rats at any of the three time points, however MRL weighed significantly more than DRL and WT rats at 60 and 90 d of age ($p<0.0001$).

BDNF protein levels in reward-related brain regions

BDNF appears to play a key role in MDD (Duman, 2002; Taliaz et al., 2010). However, whereas previous studies associated BDNF alterations with the response to stress or to antidepressants, BDNF changes have not been directly linked with the natural hereditary component of depression. Hence, we compared BDNF levels in three reward-related brain regions in DRL and MRL rats: the dorsal and ventral hippocampus (dHc and vHc, respectively) and the striatum (Str). As shown in Fig. 5, a significant effect of strain on BDNF levels was observed in the dHc ($F(2,28)=5.9$, $p=0.0072$), where *post-hoc* comparisons revealed significantly lower BDNF levels in DRL ($p=0.0029$), but not in MRL, compared with WT rats. No significant differences were observed between DRL and WT rats in the vHc and Str. BDNF levels in the vHc ($F(2,20)=7.49$, $p=0.004$) was significantly higher in MRL ($p=0.01$) compared with WT rats. BDNF levels in Str were similar in all rats.

Responses to antidepressant treatment

Behavioral responses

We continued to study whether the depressed-like behavior of DRL rats can be normalized by antidepressant treatments which are successfully used in animal models (Grant and Weiss, 2001; Will et al., 2003; Gersner et al., 2010). Following the regular battery of behavioral tests, three groups of DRL rats received 15 d of chronic

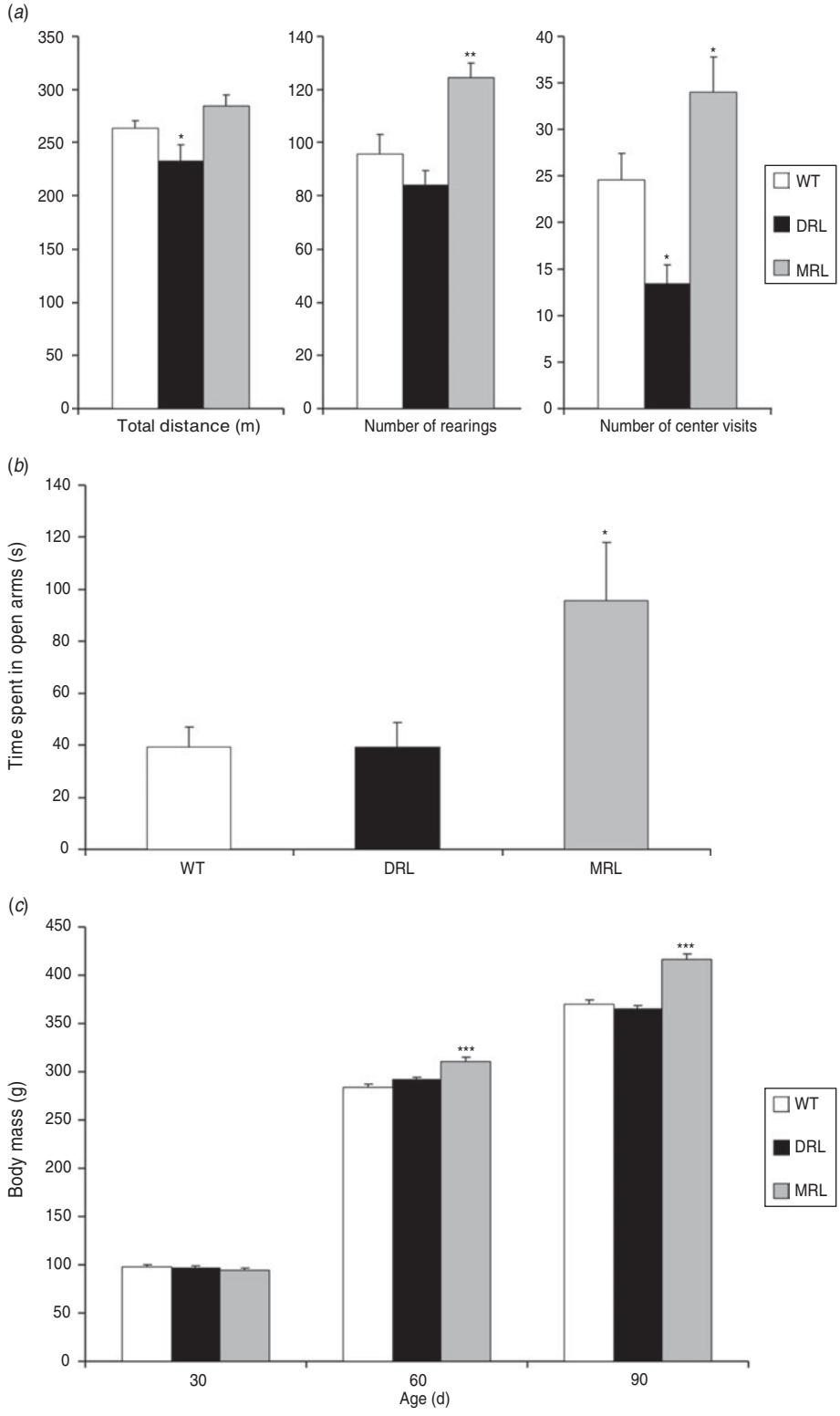

Fig. 4. Indirect effects of selective breeding. Rats from late generations of the 'depressed' and 'motivated' lines (DRL and MRL, respectively) were tested for three indirect effects of selection: (*a*) exploration of a novel environment, (*b*) elevated plus maze, and (*c*) body mass at three time points post-partum. Data presented as mean±S.E.M. *$p<0.05$, **$p<0.01$, ***$p<0.001$ for MRL and DRL *vs.* wild-type (WT). $n=10$–19 for each group in *a* and *b*, and 25–40 for each group in *c*.

treatment with the antidepressant drugs desipramine (15 mg/kg) or fluoxetine (10 mg/kg), or with saline in controls, whereas two other groups received 10 daily sessions of electroconvulsive shocks (ECS) or a corresponding sham treatment. FST mobility, SP and HCL were again evaluated following each treatment (Fig. 6*a*–*c*).

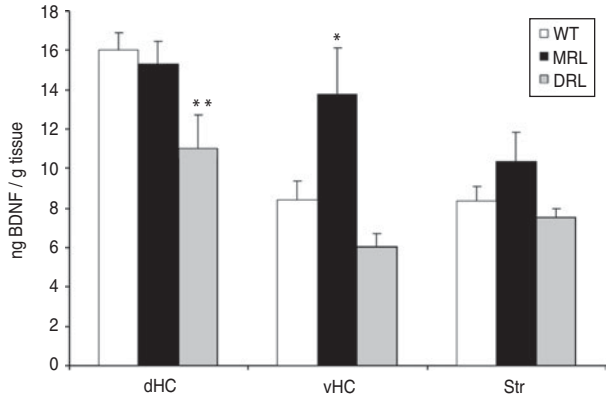

Fig. 5. Selective breeding differentially affects brain-derived neurotrophic factor (BDNF) expression in reward-related brain regions. Some individuals from generations S5 to S8, which were not selected as fathers for next generations, were sacrificed and their BDNF protein levels were determined in three brain regions: dorsal hippocampus (dHC), ventral hippocampus (vHC) and striatum (Str). Data (means±S.E.M ng BDNF/g tissue) were pooled from rats of the different generations to allow statistical analysis (n=8–15 rats in each group). *p<0.05, **p<0.01, ***p<0.001 for motivated rate line (MRL)/depressed rat line (DRL) *vs.* wild-type (WT) rats.

A significant effect was found for treatment in all behavioral assays (FST: $F(3, 37)=8.35$, $p=0.0002$; SP: $F(3, 53)=6.57$, $p=0.0007$; HCL: $F(3, 52)=2.86$, $p=0.046$) (Fig. 6*a–c*). A *post-hoc* analysis showed that, whereas neither desipramine nor fluoxetine affected the behavior of DRL rats, ECS increased all behavioral parameters (FST: $p=0.0001$; SP: $p=0.0001$; HCL: $p=0.0337$) relative to the control group.

Neurochemical responses

We also tested whether the behavioral observations are reflected molecularly in reward-related brain regions of DRL rats treated with desipramine or ECS (Table 1). This analysis shows that ECS but not desipramine markedly elevated BDNF levels ($p=0.0084$) in the dHc ($F(2, 17)=4.68$, $p=0.024$) but not in the vHc or Str, corresponding with both the aforementioned molecular characterization (Fig. 5) and behavioral observations (Fig. 6*a–c*).

Discussion

This study is the first to present a 'depressed' rat line generated through multifactorial selective breeding for three different depression-related assays. Our results indicate that BDNF changes previously associated with stress-induced depression and with response to antidepressant treatments (Duman, 2002; Toth et al., 2008; Chourbaji et al., 2011), are inherited together with depressive-like behaviors. Finally, the DRL presented in this study is responsive to ECT but not to two different pharmacological treatments, suggesting that it may model a 'medication-resistant' depression.

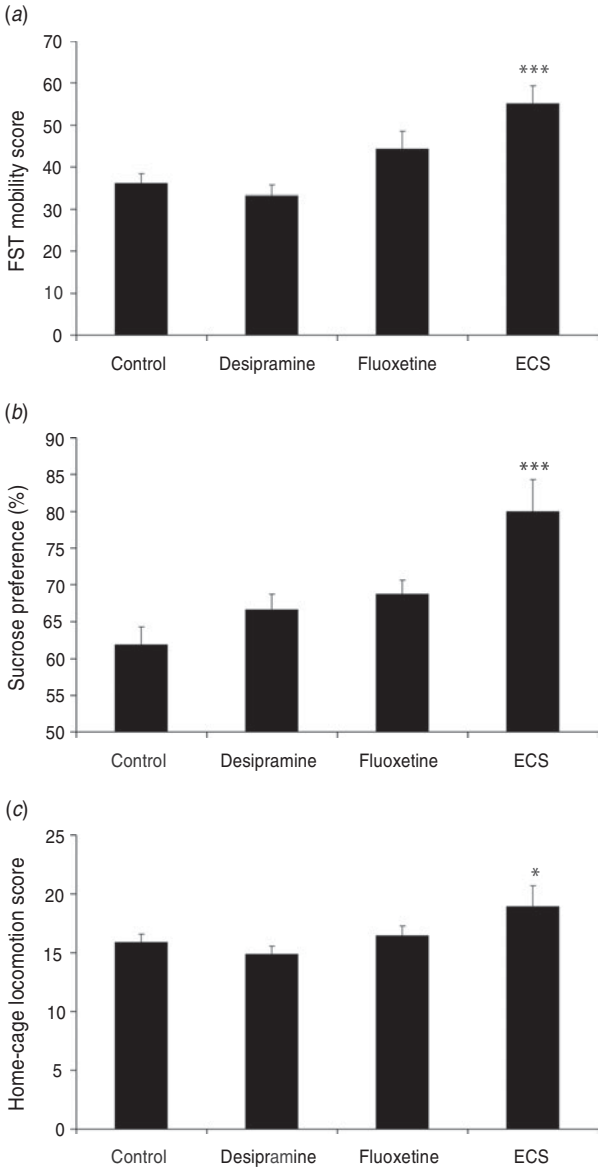

Fig. 6. Electroconvulsive shocks (ECS) but not desipramine or fluoxetine normalizes depressive-like behavior in depressed rat line (DRL) rats. Some DRL individuals from generations S5 and on, which were not selected as fathers for next generations, were used to assess the effect of three antidepressant treatments on behavior. Data show the mean (±S.E.M) behavioral scores following antidepressant treatment in the forced swim test (FST) (*a*), SP test (*b*) and home-cage locomotion test (*c*) (see text for details). n=10–22 for each group. (*d*) Mean (±S.E.M) brain-derived neurotrophic factor (BDNF) levels in the dorsal hippocampus (dHC), ventral hippocampus (vHC) and striatum (Str). n=5–11 for each group. *p<0.05, **p<0.01, ***p<0.001 *vs.* the respective control group.

The behavioral components of inherited depression

Of the three behavioral assays used here for the artificial selection procedure, decreased performance in the FST was found to be the most inheritable and decreased performance in the HCL test was found to be the least

Table 1. Electroconvulsive shocks (ECS) but not desipramine normalizes brain-derived neurotrophic factor (BDNF) levels in depressed rat lines (DRL) rats. Some DRL individuals from generations S7 and S8, which were not selected as fathers for next generations, were used to assess the effect of desipramine and ECS on BDNF levels. Data show the mean (±S.E.M) BDNF levels (in ng/g tissue) in the dorsal hippocampus (dHC), ventral hippocampus (vHC) and striatum (Str). n=5–11 for each group

	Dorsal hippocampus	Ventral hippocampus	Striatum
Control	12.01±2.37	5.96±0.57	7.81±0.67
Desipramine	13.26±1.21	6.25±0.84	6.99±1.21
ECS	**29.43±8.26****	4.96±0.76	9.59±2.1

** $p<0.01$ compared with the respective control.

inheritable component. Thus, DRL rats were born expressing depressive-like phenotypes in the FST and SP test for 10 consecutive generations, which did not change after removing the HCL component from the selection index (from generation 8 onward). Importantly, we also found that decreased performance in the OFT, a common measure of anxiety (Crawley et al., 1997; Verma et al., 2010), was indirectly inherited together with the directly selected depressive-like traits. Indeed, depression is often related to anxiety in rodents (Kalueff et al., 2007) as well as in humans (Fava and Kendler, 2000).

Our bi-directional selective breeding procedure resulted in an asymmetric behavioral response: although DRL rats exhibited a stable decrease in FST and SP performance across generations, the behavioral phenotype of MRL was not consistently different from that of WT rats in any of the three measurements used for selection. As the selective pressure for FST and SP was similar in DRL and MRL rats (Fig. 3), inherited components of the selection process seem to be decreased but not increased performance in the FST and SP test Such asymmetry was also suggested previously for FST (Weiss et al., 1998) and may reflect an 'evolutionary ceiling effect': the presumably high fitness benefits of higher 'motivation' (reflected by the FST) and higher 'hedonics' (reflected by SP) may have resulted, throughout the evolution of the species, in contemporary WT rats exhibiting naturally high 'motivational' and 'hedonic' levels. Such traits may thus be more likely to decrease than increase owing to artificial selection based on behavioral performance. MRL rats, conversely, showed indirect effects of selection manifested in the prolonged exploration of a novel environment and prolonged stay in the open arms of the EPM. Although these behaviors may potentially indicate a general increase in locomotor activity, this is unlikely because these rats did not show increased locomotor activity in the HCL assay. Thus, these phenotypes appear to be more associated with decreased anxiety (American Psychiatric Association, 2000), which may lack an evolutionary 'ceiling effect' since the costs and benefits of decreased anxiety are considerably more variable than those of decreased motivation and hedonics. Whether MRL rats show increased 'motivation' or decreased 'anxiety', however, remains to be directly tested in future studies.

Inherited changes in BDNF levels

The decreased performance in the FST and SP test was accompanied by a reduction in BDNF levels in the dorsal hippocampus. This supports the neurotrophic hypothesis of depression (Duman and Monteggia, 2006), according to which decreased hippocampal BDNF levels are associated with depression (Nibuya et al., 1995; Shirayama et al., 2002; Dwivedi et al., 2003; Karege et al., 2005; Taliaz et al., 2010) whereas increased BDNF levels have antidepressant properties (Nibuya et al., 1995; Monteggia et al., 2007; Taliaz et al., 2012). For example, chronic stress is implicated as a risk factor for depression (McGonagle and Kessler, 1990; Kendler et al., 1999) and decreases dorsal (but not ventral) hippocampal BDNF levels (Smith et al., 1995; Toth et al., 2008). Unlike the DRL rats, BDNF levels in MRL rats were not altered in the dorsal hippocampus but were increased in the ventral hippocampus. The potential relevance of BDNF in the ventral hippocampus to the behavioral phenotypes of MRL rats will need to be investigated with localized BDNF knockdown (Taliaz et al., 2010). Although beyond the scope of this study, it will be very interesting to examine the levels of BDNF, as well as of other molecular determinants (e.g. Trk B), also elsewhere in the brain and perform localized knockdown and overexpression studies to examine whether these neurochemical alterations cause the behavioral alterations in DRL or MRL rats.

Response to treatment

DRL rats were resistant to treatment with two antidepressant drugs but effectively responded to ECS, which also increased BDNF levels specifically in the dorsal hippocampus, as was demonstrated in previous studies (Nibuya et al., 1995; Altar et al., 2004). In humans, approximately 30% of MDD patients do not respond adequately to standard medications (Shelton et al., 2010), whereas 80% of these 'medication-resistant' MDD patients do respond to ECT (Kennedy and Giacobbe, 2007). It is therefore possible that DRL is a generally 'medication-resistant' line.

Conclusions

To conclude, this study establishes a novel line of rats obtained through a multifactorial selective breeding procedure. Rats in this line express, since birth, decreased performance in the forced swimming test together with decreased sucrose preference (two directly selected traits)

and decreased exploration of a novel arena (an indirectly selected trait). Consistent with the neurotrophic hypothesis of depression, these inheritable phenotypes are also reflected molecularly by decreased BDNF levels in the dorsal hippocampus. Electroconvulsive shock therapy but not pharmacological treatment normalizes both the behavioral phenotypes and the BDNF changes in DRL rats, suggesting that these rats may be used to differentiate the efficacy of antidepressant strategies, as well as to evaluate the underlying mechanisms.

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Statement of Interest

None.

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