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Prelimbic Stimulation Ameliorates Depressive-Like Behaviors and Increases Regional BDNF Expression in a Novel Drug-Resistant Animal Model of Depression

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ABSTRACT

Background: Approximately one third of all major depression patients fail to respond to conventional pharmacological antidepressants, and brain stimulation methods pose a promising alternative for this population. Recently, based on repeated multifactorial selective inbreeding of rats for depressive-like behaviors, we introduced a novel animal model for MDD. Rats from this Depressive Rat Line (DRL) exhibit inherent depressive-like behaviors, which are correlated with lower levels of brain-derived neurotrophic factor (BDNF) in specific brain regions. In addition, DRL rats do not respond to antidepressant medication but respond to electroconvulsive treatment, and they can thus be utilized to test the effectiveness of brain stimulation on hereditary, medication-resistant depressive-like behaviors.
Objective: To test the effect of sub-convulsive electrical stimulation (SCES) of the prelimbic cortex, using TMS-like temporal pattern of stimulation, on depressive-like behaviors and regional BDNF levels in DRL rats.
Methods: SCES sessions were administered daily for 10 days through chronically implanted electrodes. Temporal stimulation parameters were similar to those used in TMS for major depression in human patients. Depressive-like behaviors were assayed after treatment, followed by brain extraction and regional BDNF measurements.
Results: SCES normalized both the depressive-like behaviors and the reduced BDNF levels observed in DRL rats. Correlation analyses suggest that changes in specific behaviors are mediated, at least in part, by BDNF expression in reward-related brain regions.
Conclusions: Brain stimulation is effective in a drug-resistant, inherited animal model for depression. BDNF alterations in specific regions may mediate different antidepressant effects.

Introduction

Major depressive disorder (MDD) is a chronic brain disease characterized by several co-occurring behavioral symptoms, including anhedonia, loss of interest and reduced motivation, fatigue, sleep disturbances, and more [1]. Although environmental factors often trigger MDD, the heritability of this disease is estimated at 40–50% [2]. Despite extensive attempts to improve antidepressant treatment strategies during the past decades, about 30% of all MDD patients are still considered "drug-resistant" and do not respond to pharmacotherapy, while many others refrain from pharmacotherapy due to its considerable side effects [3–6]. Brain-stimulation techniques can reduce depressive symptoms and have relatively high response rates in drug-resistant MDD patients [7–15], although some techniques have substantial side effects and other caveats [16–18].

Albeit the obvious differences between humans and animal models, preclinical research can help evaluate potential mechanisms and spatiotemporal parameters of brain stimulation treatments [19–21] and, accordingly, several studies evaluated effects of brain stimulation on conventional animal models for MDD [22–26]. To induce the depressive-like state, most studies employed the widely used chronic mild stress (CMS) paradigms [27]; this approach, however, completely lacks the hereditable component of depression (which may be intimately involved in the translational aspects of the treatment [2,28]) and does not model "drug-resistant" MDD [29,30].

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By selectively inbreeding rats that show a multifactorial depressive-like behavior, our group has recently developed the "Depressed Rat Line" (DRL) model for MDD [31]. The phenotype of DRL rats includes inherent depressive-like behavioral symptoms accompanied by a significant reduction in hippocampal brain-derived neurotrophic factor (BDNF) levels, a phenomenon that was suggested to correlate with and even cause depression in both animal models and humans [32–35]. Thus, DRL rats appear to model hereditable components of MDD. Moreover, DRL rats do not respond to pharmacological antidepressants (namely, to fluoxetine or desipramine), although they do respond to electroconvulsive therapy (ECT); in that respect, DRL rats appear to model, at least to some extent, drug-resistant MDD.

Here, we studied the phenotypic (behavioral and BDNF-related) alterations in DRL rats treated with repeated sub-convulsive electrical stimulations (SCES) of the prelimbic cortex (PLC), delivered through chronically implanted electrodes. Unlike deep-brain stimulation (DBS), which is applied at relatively high frequencies (100–140 Hz) and is based on a continuous (24 h/day, 7 days/week) interference with circuit activity [36], we focused on the long-term, plasticity-related effects of a relatively low-frequency (20 Hz) stimulation trains applied repeatedly for a short period (10 min/day for 10 days). We employed a temporal stimulation pattern resembling the pattern used for repeated transcranial magnetic stimulation (rTMS) treatments of MDD [37–41]; this pattern is suggested to induce long-lasting neuroplastic alterations [42] that can effectively treat MDD in moderately drug-resistant patients [38–41], although it is considerably less effective in extremely drug-resistant or psychotic-depressive patients [38,43]. An 'rTMS-like' SCES pattern has previously been shown to reduce depressive-like symptoms in wild-type rats subjected to CMS [24], but it was never tested in a drug-resistant, hereditary model of MDD.

Because ECT in DRL rats has been shown to ameliorate depressive-like behaviors and increase hippocampal BDNF levels [31], and because PLC SCES in rats submitted to CMS has been shown to ameliorate depressive-like behaviors and increase hippocampal and striatal BDNF levels [24], we hypothesized that a localized sub-convulsive PLC stimulation (PLC SCES) in DRL rats will be sufficient to ameliorate depressive-like behaviors and affect BDNF expression.

Materials and methods

Experimental design and animals

We tested behavioral and BDNF responses to treatment with repeated SCES to the PLC in adult (300 gr) male DRL and wild-type (WT) Sprague-Dawley rats (N = 18–19 in each group). All rats were implanted with a SCES electrode (see below) and allowed to recover for one week, after which they were treated for 10 consecutive days (10 min/day) with either real or sham SCES (Fig. 1). Then, during the three weeks following the SCES treatment, the rats underwent a series of behavioral assays (see details below) in the following order: Sucrose Preference Assay (SPA), Home Cage Locomotion Assay (HCLA), exploration of a novel environment (Exploration assay), and Forced Swim Test (FST). To avoid the short-term effect of FST-induced stress [44], all rats were allowed three days of resting before sacrificing them and analyzing their brains for BDNF levels. All behavioral assays and BDNF analyses were blind to subject group identity.

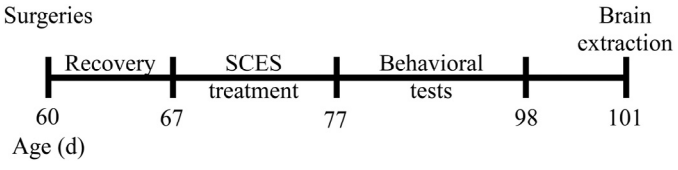

Figure 1. Experimental timeline. Sixty-days-old male Sprague-Dawley WT and DRL rats (N = 18–19 in each group) were implanted with a SCES electrode. After one week of recovery, 10-min long SCES sessions were administered daily for 10 consecutive days. During the following three weeks, all rats underwent behavioral assays in the following order: Sucrose Preference Assay (SPA), Home Cage Locomotion Assay (HCLA), exploration of a novel environment, and Forced Swim Test (FST). Three days after the FST, the rats were sacrificed and their brains were removed for a BDNF ELISA.

All rats were singly housed and maintained in a 12 h light/12 h dark cycle with food and water provided *ad libitum*. All treatments and behavioral assays were conducted at approximately the middle of the dark phase of the cycle (an exception to this was the home cage locomotion assay, which was monitored continuously). DRL rats were bred in our animal facilities (see below); WT rats were purchased from Harlan, Israel, and were allowed 2 weeks of habituation to the animal facility prior to surgery, treatment and behavioral assays. All rats were handled and all experiments were conducted according to the guidelines of the Committee for the Ethical Care and Use of Animals in Research (CECUAR) of Ben-Gurion University of the Negev (Beer-Sheva, Israel), which are in complete accordance with the NIH guidelines for care and use of laboratory animals.

The DRL rats used in this study were direct decedents of the rats used in our previous report [31], which originated from a pair of WT Sprague-Dawley parents. We continued to inbreed those rats, namely, by artificially selecting for depressive-like behavioral phenotypes as detailed in reference 31, such that all rats used in the current study were of generations S18–S23. To verify that DRL rats of generations S18–S23 show drug-resistant depressive-like phenotypes similar to those of the earlier (S5–S10) generations – we also tested the behavioral and BDNF-related response of some of the S18–S23 DRL rats to fluoxetine. The relevant methodology and findings of those experiments are detailed in the Supplementary Material of this article.

Electrode implantation and SCES treatment

We followed the protocol detailed and explained in reference 24. Briefly, we implanted anesthetized rats with a stimulating electrode (Plastics One, Roanoke, VA) in the left PLC (coordinates in millimeters relative to bregma: +3.7 anteroposterior, –0.4 mediolateral, +3.5 dorsoventral from scalp level). Immediately following the surgery, we injected rats subcutaneously with Neurocarp (Pfizer; 0.03 ml of 50 mg/ml carprofen) and applied V-Dalgin (Dipyron) to their drinking water for 4 days thereafter (1.2 ml in 250 ml of drinking water). Experiments began 7 days after electrode implantation. Because we extracted tissue punches from the PLC for BDNF analyses at the end of the experiments (see below), it was impossible to verify electrode locations histologically. However, given our extensive experience in electrode implantation into the PLC (in reference 24, for instance, histological analyses verified that more than 90% of our PLC-implanted electrodes were located appropriately), we assume that most electrodes were in the PLC.

The stimulation regime included daily ten-minute sessions administered for 10 consecutive days. In each session, the implanted electrode was connected with a flexible wire to an electrical stimulator and the rat was then placed in its home cage. As detailed in reference 24, we used a TMS-like temporal pattern of pulses, such that each stimulation cycle comprised 100 pulses (pulse width = 0.2 msec; pulse intensity = 400 μA) applied during a 5-s period (i.e., at 20 Hz), followed by a 20-s inter-train interval (pause). Sham-treated controls underwent the same surgery and handling protocol, but no current was applied through the electrode.

Behavioral analyses

To evaluate depressive-like behaviors, we used well-established paradigms, as we detailed elsewhere [31]. In the SPA, the consumption of a 0.2% sucrose solution as percentage of total liquid consumption was calculated daily for each rat and averaged over 10 days to determine sucrose preference. In the HCLA, locomotion was calculated as the average overall distance traveled in the home cage during three consecutive nights. In the Exploration assay, the distance traveled, number of rearings, and number of visits in the central region of a 40×40 cm exploration box were monitored automatically via interruption of photo beams. In the forced swim test (FST), the rat was placed in a custom-built cylindrical tank (40 cm high and 18 cm in diameter) for a single 5-min session, its behavior was monitored, and its swimming and immobility time were later extracted and analyzed with a software developed in our laboratory [45,46].

Biochemical analyses

The complete protocols for tissue punches, protein extraction, and BDNF ELISA are described in our previous works [31,47]. We extracted the brains three days following the last behavioral assay and used a sandwich ELISA to analyze BDNF concentrations (normalized to tissue weight) in bilateral tissue punches obtained from the prelimbic cortex (PLC), nucleus accumbens (NAc), striatum, and dorsal and ventral hippocampus (dHC and vHC, respectively), as detailed in Fig. S1. Because SCES was applied unilaterally to the left PLC, we separated the left and right PLC (lPLC and rPLC, respectively) for the BDNF analysis.

Statistical analyses

Significance of treatment effect was analyzed with a two-way analysis of variance (ANOVA) model, with Strain (DRL/WT) and Treatment (SCES/Sham) as the independent variables, and behavioral score or BDNF levels in each brain region as the dependent variables. When significant main effects or interactions were found, a Fisher's post-hoc test was conducted. Data distributions for all ANOVA analyses were tested for normality by using the Kolmogorov–Smirnov test.

A backward stepwise regression procedure was used to select the brain regions wherein BDNF levels best predict behavioral scores. We began with a complete regression model, in which BDNF levels in seven brain regions were used as predictors of behavior, and gradually subtracted variables that did not significantly contribute to the model. The final model was selected when no more variables could be subtracted without degrading the model.

A mediation model was used to test whether the independent variables may be mediated by BDNF levels in certain brain regions (see Results). Because we were interested in evaluating whether several independent variables (strain, treatment, and their interaction) exert a conditional mediation influence on the dependent variable (behavior), the commonly used Sobel test [48] —which regards simple mediating models, wherein one independent variable exerts its influence on one dependent variable through one mediating variable – was not suitable for the current study; instead, we employed the principles suggested previously by Baronand Kenny [49]. Briefly, the procedure included comparing a full model to a reduced model, as follows: to construct the full model, we used a two-way ANOVA with Strain (DRL/WT) and Treatment (SCES/Sham) as independent variables and with behavioral scores as a dependent variable. Then, to construct the reduced model, we first calculated the simple linear regression for each strain, predicting the behavioral score by the BDNF level. Then, we constructed a set of errors, defined as the numerical difference between each observed score and the corresponding theoretical score predicted by the linear model. Thus, this set of errors essentially reflects the 'clean' behavioral scores in which the influence of BDNF levels on behavior is omitted. Finally, we performed a second ANOVA on these 'clean' scores and compared the results to the full ANOVA model. For correlation analyses, we used False Discovery Rate (FDR [50]) to correct for multiple comparisons.

Results are expressed as mean ± SEM throughout the manuscript. For brevity, we focus here on statistically significant comparisons, whereas complete statistical analyses, including significant and non-significant comparisons, are detailed in the Supplementary Material of this article. All analyses were conducted with Statistica software version 8.0 (Statsoft, Tulsa, OK, USA).

Results

We have previously characterized DRL rats as a drug-resistant model of MDD [31]; however, rats used in that previous study were of early generations of the artificial selection process (namely, S5-S10), whereas those in our current study were of later generations (S18-S23). Thus, we first verified that the DRL rats used in the current study indeed show a similar inherent depressive-like phenotype that is resistant to the commonly used antidepressant fluoxetine (see Supplementary Material for details). Indeed, similar to their S5–S10 predecessors (who showed resistance to desipramine or fluoxetine treatments), S18–S23 DRL rats (N = 17), but not WT rats (N = 17), showed inherent depressive-like behavioral (Fig. S2 and Table S1) and neurochemical (Fig. S3 and Table S2) phenotypes, that were generally unresponsive to fluoxetine (a trend toward increased BDNF levels was observed in fluoxetine-treated DRL rats, but it was significant only in the dorsal hippocampus and was lower than the increase observed following SCES). Thus, we continued to elucidate the effect of repetitive SCES on the depressive-like phenotype of DRL rats.

Repetitive SCES ameliorates depressive-like behaviors in DRL rats

The 10-d SCES active (but not sham) treatment dramatically ameliorated most depressive-like behaviors in DRL rats (Fig. 2 and Table S3). A significant main effect was found for Strain (DRL versus WT rats) in the SPA ($F(1,69) = 13.27$, $p < 0.001$), FST ($F(1, 69) = 9.8$, $p < 0.01$), HCLA ($F(1,69) = 16.88$, $p < 0.001$), and number of rearings in the Exploration assay ($F(1,69) = 8.1$, $p < 0.01$). In addition, a significant main effect was found for Treatment (SCES versus sham) in the SPA ($F(1,69) = 23.01$, $p < 0.001$) and FST ($F(1, 69) = 6.34$, $p < 0.01$), and in the number of rearings ($F(1,69) = 19.1$, $p < 0.001$) and number of center visits ($F(1,69) = 9.65$, $p < 0.01$) in the Exploration assay. A significant Strain × Treatment interaction was found in the SPA ($F(1, 69) = 14.03$, $p < 0.001$) and in the number of center visits in the Exploration assay ($F(1,69) = 10.44$, $p < 0.01$). Post-hoc analyses revealed that the behavior of sham-treated DRL rats was significantly more depressive-like than that of sham-treated WT rats in the SPA ($p < 0.001$), FST ($p < 0.001$), HCLA ($p < 0.05$), and in the number of rearings ($p < 0.01$) and center visits ($p < 0.05$) in the Exploration assay. SCES-treated DRL rats showed significantly less depressive-like behavior than sham-treated DRL rats in the SPA ($p < 0.001$), FST ($p < 0.01$), and number of rearings ($p < 0.001$) and center visits ($p < 0.001$) in the Exploration assay, but not in the HCLA or in distance traveled in the Exploration assay. Notably, following 10 days of treatment, the behavior of SCES-treated DRL rats became comparable to that of sham-treated WT rats in the SPA ($p = 0.42$), FST ($p = 0.66$), and the number of center visits ($p = 0.052$) and rearings ($p = 0.27$) in the Exploration assay.

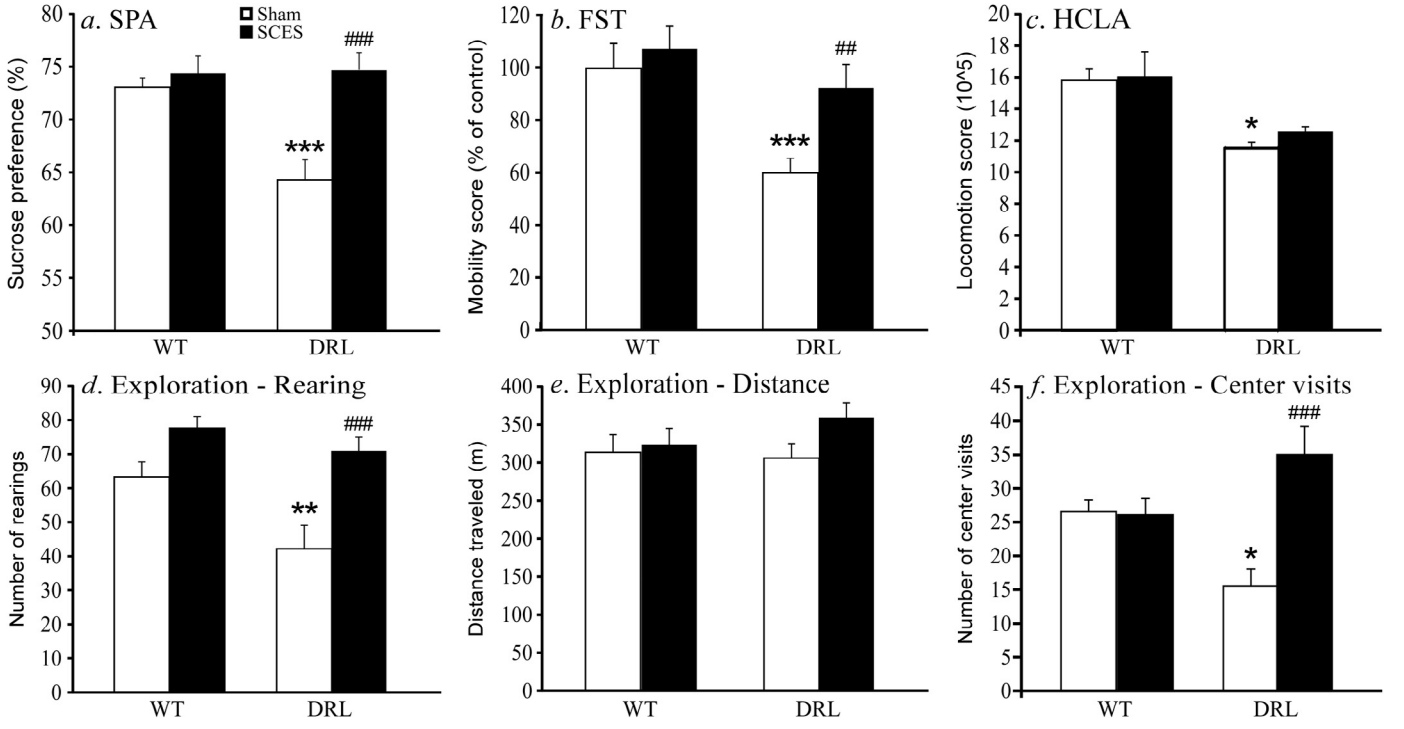

Figure 2. Repeated SCES treatment to the PLC normalizes inherent depressive-like behaviors in DRL rats. Behavioral scores of sham- and SCES-treated Depressive Rat Line (DRL) and wild-type (WT) rats (N = 18–19 per group) in (*a*) the Sucrose Preference Assay (SPA); (*b*) the Forced Swim Test (FST); (*c*) the Home Cage Locomotion Assay (HCLA); and (*d–f*) the different parameters of the Exploration assay. Sham-treated DRL rats exhibited a marked depressive-like behavior as compared with sham-treated WT rats, including a lower preference for sucrose in the SPA, a shorter swimming duration in the FST, reduced locomotion in the HCLA, a lower number of rearings and center visits in the Exploration assay. The SCES treatment ameliorated most of these depressive-like behaviors, such that the sucrose preference, swimming duration, rearing activity, and number of center visits of SCES-treated DRL rats were significantly higher than those of sham-treated DRL rats (namely, by 16%, 57%, 68%, and 125%, respectively; N = 18–19 per group). Moreover, following the SCES treatment, the pronounced inherent depressive-like behavior of DRL rats was practically normalized and generally became comparable to that of WT rats in the aforementioned assays, but not in the HCLA. The SCES treatment had no effect on any of these behaviors in the WT rats. Bars represent means ± SEMs. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ as compared with sham-treated WT rats; $^{\#}p < 0.05$ $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ as compared with sham-treated DRL rats.

Repetitive SCES increases BDNF levels in DRL rats, in correlation with the behavioral response to the treatment

The SCES treatment induced a significant increase in BDNF protein levels within all the examined reward-related brain regions of DRL rats (Fig. 3 and Table S4). A significant main effect was found for Treatment (SCES versus sham) in the dHC ($F(1,69) = 28.93$, $p < 0.001$), vHC ($F(1,69) = 5.33$, $p < 0.05$), right PLC ($F(1,32) = 4.65$, $p < 0.05$), and striatum ($F(1, 69) = 15.23$, $p < 0.01$). A significant Strain × Treatment interaction was found in the dHC ($F(1,69) = 13.82$, $p < 0.01$), left PLC ($F(1,32) = 6.05$, $p < 0.05$), right PLC ($F(1,32) = 6.09$, $p < 0.05$), and striatum ($F(1, 69) = 16.41$, $p < 0.001$). A marginally significant interaction was found in the vHC ($F(1,69) = 3.91$, $p < 0.052$). Post-hoc analyses revealed that the BDNF levels of sham-treated DRL rats were significantly lower than those of sham-treated WT rats in the dHC ($p < 0.005$), lPLC ($p < 0.05$), rPLC ($p < 0.05$), and striatum ($p < 0.001$). In addition, BDNF levels in SCES-treated DRL rats were significantly higher than in sham-treated DRL rats in the dHC ($p < 0.001$), vHC ($p < 0.005$), lPLC ($p < 0.01$), rPLC ($p < 0.01$), and striatum ($p < 0.001$).

To further explore the association between BDNF levels and depressive-like behaviors in DRL rats, we calculated the Pearson correlation coefficients between BDNF levels and behavioral scores in the SPA and FST, wherein the depressive-like behavior of DRL rats was most pronounced (Table 1, Fig. 4). These analyses showed a significant linear correlation between behavioral score in the SPA and BDNF levels in the dHC, striatum, rPLC and vHC; and between behavioral score in the FST and BDNF levels in the rPLC, lPLC, vHC and dHC (brain areas are denoted by descending order of association magnitude). No such correlations were found in WT rats, suggesting that BDNF levels may mediate depressive-like behaviors in DRL rats, as well as the effect of SCES on those behaviors. To test this possibility, we used a mediation model, as described below.

A mediation model indicates strong relationship between BDNF levels in the dHC or rPLC and the effect of strain and treatment on SPA and FST, respectively

We investigated whether BDNF levels may mediate (1) the behavioral differences between DRL and WT rats; and (2) the differential behavioral effects of the SCES treatment. Here, again, we focused on the SPA and FST because behavioral differences in these assays were the most pronounced and because these assays are most often used to assess depressive-like behaviors in animal models. As a first step, we used a backward stepwise regression procedure (see Materials and Methods) to select the brain regions wherein BDNF levels best predict behavioral scores in each of these assays. The stepwise selection algorithm concluded in two final models, wherein BDNF levels in the dHC were the sole predictor of behavioral score in the SPA, and BDNF levels in the rPLC were the sole predictor of behavioral score in the FST. All other six predictors (i.e., BDNF levels in each of the other brain regions tested) were redundant, such that excluding them from the model did not significantly decrease prediction power.

To test whether the effect of Strain and Treatment on the behavioral scores in the SPA and FST may be mediated by BDNF levels in the dHC and rPLC, respectively, we constructed a mediation model

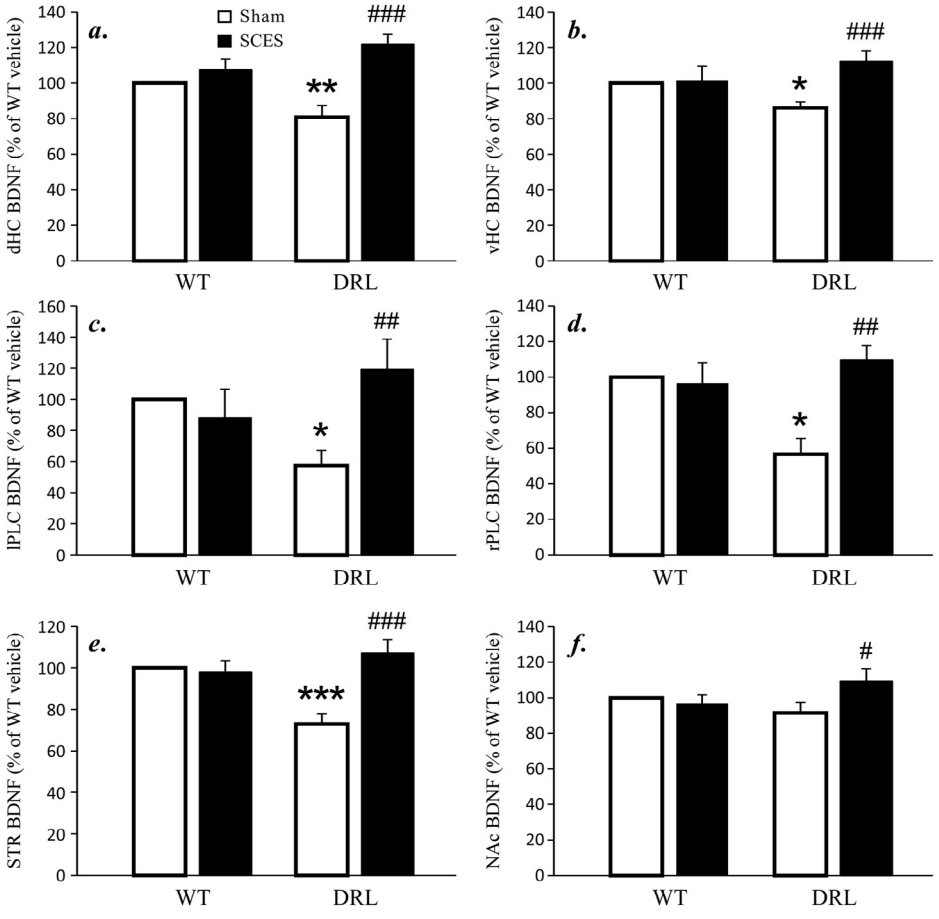

Figure 3. Repeated SCES treatment to the PLC normalizes BDNF levels in DRL rats. ELISA measurements of BDNF levels in the (*a*) dorsal hippocampus (dHC); (*b*) ventral hippocampus (vHC); (*c*) left prelimbic cortex (left PLC); (*d*) right prelimbic cortex (right PLC); (*e*) striatum; and (*f*) nucleus accumbens (NAc) of sham- and SCES-treated Depressive Rat Line (DRL) and wild-type (WT) rats (N = 18–19 per group). A comparison between the sham-treated groups revealed that BDNF levels in DRL rats were significantly lower than those in their WT counterparts in most brain regions examined, including the dHC, vHC, left PLC, right PLC, and striatum (namely, 20%, 16%, 42%, 43% and 27% lower, respectively), but not in the NAc. The SCES treatment significantly increased BDNF levels in DRL rats, as compared with their sham-treated counterparts, in the dHC (50% increase), vHC (30% increase), lPLC (107% increase), rPLC (93% increase), and striatum (46% increase). Bars represent mean ± SEM of BDNF levels as percent of those levels in sham-treated WT rats. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ as compared with sham-treated WT rats; $^{\#}p < 0.05$ $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ as compared with sham-treated DRL rats.

(Fig. 5; see Materials and Methods). This model assumes that, if the level of BDNF in the dHC (for the SPA) and rPLC (for the FST) indeed serves as a mediating factor of behavior, then statistically eliminating the influence that this factor has on behavior would decrease the magnitude of the observed associations between behavioral score and Strain and Treatment. Indeed, for the SPA, the full ANOVA model explained 44% of the variance in behavioral scores and was highly significant ($F(3,69) = 18.08$, $p < 0.001$), but statistically eliminating the effect of dHC BDNF levels resulted in an ANOVA model that only explained 5% of the variance, and was not significant ($F(3,69) = 1.21$, $p = 0.31$). In this reduced model, behavioral score was not affected by either Strain ($F(1,69) = 0.0$, $p = 0.98$), Treatment ($F(1,69) = 3.34$, $p = 0.07$), or the Strain × Treatment interaction ($F(1,69) = 0.14$, $p = 0.7$). A similar result was found for the effect of rPLC BDNF levels on behavioral scores in the FST. The full model explained 22% of the behavioral variability and was highly significant ($F(3,31) = 6.35$, $p < 0.001$), whereas eliminating the effect of rPLC BDNF levels resulted in a model that explained only 4% of the variability and was not significant ($F(3,31) = 0.45$, $p = 0.71$). This reduced model was also not affected by either Strain ($F(1,31) = 0.0$, $p = 0.96$), Treatment ($F(1,31) = 1.05$, $p = 0.31$), or the Strain × Treatment interaction ($F(1,31) = 0.21$, $p = 0.64$). Taken together, these findings strongly suggest the BDNF levels in the dHC and rPLC play a major role in mediating the effects of both strain and treatment on depressive-like behaviors.

Table 1
Pearson correlation coefficients between BDNF levels in specific brain regions and behavioral scores in the Sucrose Preference Assay (SPA) and in the Forced Swim Test (FST).

		VHc	DHc	rPLC	lPLC	Str	Nac
DRL	SPA	0.44**	0.72***	0.66**	0.41	0.65***	0.31
	FST	0.42**	0.37	0.59**	0.58*	0.21	–0.1
WT	SPA	0.09	–0.2	–0.25	0.69	–0.4	0.13
	FST	0.03	0.12	0.45	–0.21	–0.08	0.32

Abbreviation as in Figure 3. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Discussion

A major finding in our study is that SCES of the PLC, applied in short daily sessions for 10 consecutive days, dramatically ameliorates most depressive-like behaviors in a hereditary rat model of drug-resistant MDD, along with significant alterations in regional BDNF expression. We used a unilateral left stimulation in light of previous studies in MDD patients [51,52] and in animal models [24], but the effects of bilateral or unilateral right stimulation might

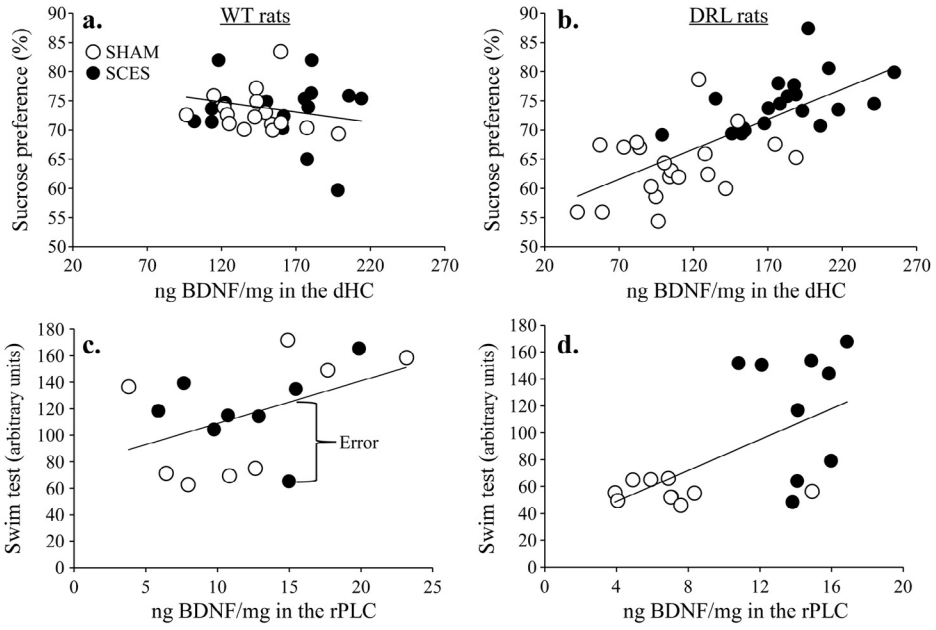

Figure 4. Simple linear regression models predicting behavioral scores in the SPA and FST by BDNF levels in the dHC (*a, b*) and rPLC (*c, d*), respectively. Abbreviations as in previous figures. Filled and empty circles indicate SCES and sham treatment, respectively. Behavioral scores in the SPA (*a, b*) and in the FST (*c, d*) were correlated with dHC (*a, b*) and rPLC (*c, d*) BDNF levels in DRL rats (*a, c*) but not in WT rats (*b, d*). "Error" in (*c*) indicates the distance between one specific data point and the linear regression model. A set of such errors was constructed to perform the mediation model shown in Fig. 5.

warrant future research. Our stimulation protocol, which generally resembles temporal patterns used in human rTMS studies [53], was well-tolerated by the rats and exerted long-term (>30 days) behavioral and neurochemical effects. The fact that it normalized sucrose preference in DRL rats is particularly encouraging, because, of all depressive-like symptoms, this assay is considered to have the highest face validity to anhedonia [54] – which is a major symptom of MDD. It is noteworthy that the SCES treatment did not normalize the reduction in home-cage locomotion observed in DRL rats. The HCLA is often considered the animal analogue of psychomotor retardation in MDD patients – a symptom presumably related specifically to abnormal dopaminergic function (e.g., MDD patients exhibiting psychomotor retardation are responsive only to selective dopamine-reuptake inhibitors [55–57]). Thus, as compared with other depressive symptoms, psychomotor retardation does not appear to be, in itself, an optimal measurement for

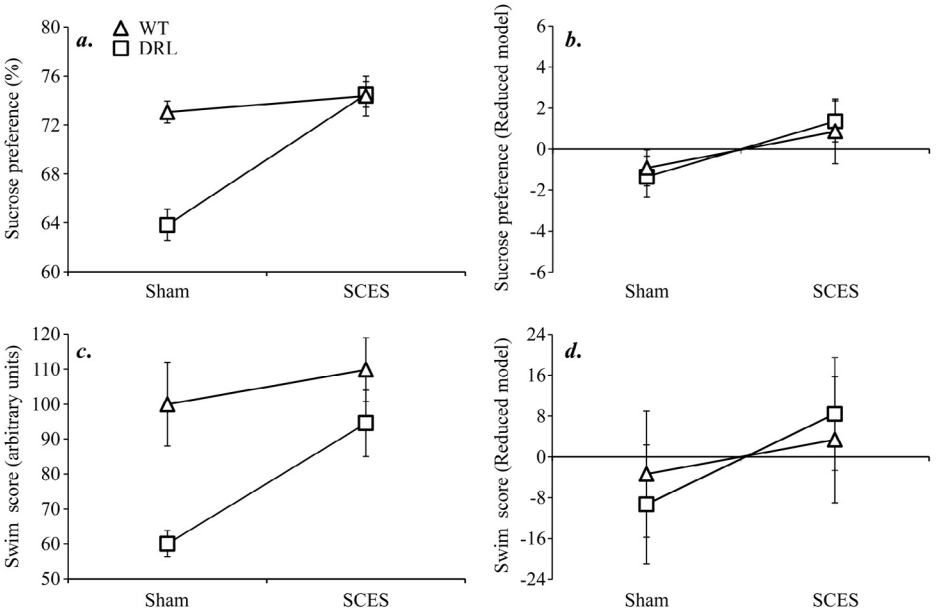

Figure 5. BDNF levels in the dHC and rPLC mediate the effect of Strain and Treatment on behavioral scores in the SPA and FST, respectively. Abbreviations as in previous figures. Triangles and squares indicate WT and DRL rats, respectively, and top (*a, b*) and bottom (*c, d*) panels show results for SPA and FST, respectively. Left panels (*a, c*) show the full ANOVA models, which were calculated based on the observed behavioral scores. Right panels (*b, d*) show the reduced ANOVA models, which were calculated on the sets of errors that were obtained by statistically omitting the effect of BDNF levels in the dHC and rPLC on the SPA and FST scores, respectively. See text for further details.

testing the efficacy of non-dopamine-related antidepressant treatments.

An important finding of our study is that BDNF levels in specific brain regions, most prominently in the ventral PLC and dHC, may mediate both the depressive-like behaviors and the effects of the PLC-SCES treatment in DRL rats. The changes in BDNF levels in DRL rats were detected more than three weeks after the last SCES session, suggesting that the treatment induced long-term changes in brain plasticity. It is possible that SCES or fluoxetine treatment also exerted a more transient effect on BDNF levels (in other brain regions of either DRL or WT rats) but, due to methodological constraints, we were blind to these changes in the current experiment and they should be examined in future studies (by sacrificing animals immediately following treatment, without comprehensive behavioral testing). Similar to the effects of SCES reported in the current study, we have previously found that ECT (but not desipramine or fluoxetine) ameliorates depressive-like behaviors in DRL rats, and that this effect is accompanied by an increase in dHC BDNF expression. However, unlike in the current study, the amelioration of depressive-like behaviors in ECT-treated DRL rats was not accompanied by changes in vHC and striatal BDNF levels [31], possibly highlighting the importance of increasing BDNF levels in the dHC (rather than in the vHC or striatum) for the antidepressant-like effects of brain stimulation in DRL rats; unfortunately, PLC BDNF levels were not evaluated in our previous ECT study. Similar long-term beneficial effects of SCES on depressive-like behaviors have been correlated with normalization of BDNF levels in the dHC (and in the striatum) also in the CMS model of depression [24]. Taken together, these findings are in line with the neurotrophic hypothesis of depression, which postulates that a reduction in BDNF levels in specific brain areas is critical to the impaired neuroplasticity associated with MDD [35,58–62] and that normalization of BDNF expression is essential for the effectiveness of antidepressant treatments [24,25,32,47,59,63–65]. It is not entirely clear how BDNF affects depressive-like symptoms, but it is plausible that adult hippocampal neurogenesis plays a role in recovery [66], e.g., through interactions between BDNF and the serotoninergic receptor 5-HT1A [66,67]. As dHC BDNF levels in DRL rats in the current study were increased following the SCES treatment, future experiments that will evaluate hippocampal neurogenesis in this model may further contribute to our understanding of the mechanism underlying drug-resistant MDD. In addition, our statistical analyses support a full mediation model wherein BDNF levels in the dHC and rPLC explain both the strain- and treatment-related behavioral variability in sucrose preference and in swimming duration, respectively. It is noteworthy that fluoxetine also somewhat increased BDNF levels in DRL rats, an increase that was significant only in the dHC and was not accompanied by a behavioral effect. Although there was a trend for higher BDNF levels in fluoxetine-treated DRL rats, this increase was very small and localized as compared with the robust and widespread BDNF increase in SCES-treated rats. It is very plausible that the small increase in BDNF levels in the dHC of fluoxetine-treated rats was not sufficient to be manifested in behavioral changes, either because it was too small in itself or because it was not accompanied by a significant BDNF increase in other brain regions, most probably in the PLC (e.g., lack of a possible synergistic effect). Direct manipulation of BDNF (e.g., BDNF knockdown in specific brain regions) in DRL rats may prove a causal relationship between regional BDNF levels, specific depressive-like behaviors, and treatment efficacy; however, such manipulations were beyond the scope of the current study. Yet, if factors other than BDNF are directly responsible for the observed behavioral and neurochemical traits of DRL rats, then those factors can be expected to correlate strongly with BDNF levels and will probably belong to the same functional system.

Conclusions

The DRL model can serve as substrate for elucidating neurobiological mechanisms underlying inherited depressive-like behavior that is, at least in part, resistant to antidepressant medications. Therefore, this model can assist in the development and optimization of novel therapeutic approaches for "drug-resistant" MDD patients. Notwithstanding the obvious differences between animal models and the human conditions that they were designed to mimic, the data provided here suggest that multiple stimulation sessions targeting the prefrontal cortex can be effective in treating drug-resistant and genetically predisposed MDD patients. Furthermore, our findings indicate that such treatments may alleviate specific symptoms of depression by normalizing BDNF levels in the hippocampus and locally in the prefrontal cortex.

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Appendix. Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.brs.2015.10.009.

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