Altered Brain-Derived Neurotrophic Factor Expression in the Ventral Tegmental Area, but not in the Hippocampus, Is Essential for Antidepressant-Like Effects of Electroconvulsive Therapy

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**Background:** Impaired neuronal plasticity and, specifically, altered expression of brain-derived neurotrophic factor (BDNF) were shown to play a critical role in depressive behavior and the mechanism of various antidepressant treatments including electroconvulsive therapy (ECT). Interestingly, opposing roles were suggested for BDNF in the hippocampus and the ventral tegmental area (VTA), while interactions between these regions were shown on various levels. Here, we evaluated whether BDNF plays an essential role in the antidepressant-like effects of ECT and performed a direct comparison between BDNF manipulations in the VTA and the hippocampus.

**Methods:** Knockdown or overexpression of BDNF was induced in hippocampus or VTA of rats by microinjection of specific lentiviral vectors. The effects of these manipulations on antidepressant outcomes of ECT were evaluated by the forced swim test and by sucrose preference measurements, and BDNF expression levels were assessed in other reward-related brain regions.

**Results:** Here, we show that whereas ECT increased hippocampal BDNF expression, induction of hippocampal BDNF knockdown did not block its antidepressant-like effect. Importantly, we found that ECT caused a robust reduction in VTA BDNF levels. Moreover, VTA BDNF knockdown alone was sufficient to induce an antidepressant-like effect, and VTA BDNF overexpression blocked the antidepressant-like effect of ECT.

**Conclusions:** While neuroplastic alterations, as expressed by changes in BDNF expression within different brain regions, are induced by ECT, the antidepressant-like effect of ECT in an animal model depends on reduction of VTA BDNF expression but not on the elevation of hippocampal BDNF expression.

**Key Words:** BDNF, depression, ECT, hippocampus, rat model, VTA

Depression is a life threatening illness with a lifetime prevalence of about 17% (1). Forty to fifty percent of the risk for depression is genetic (2); however, the specific genes involved and their altered expression in relevant brain sites or their role in the effectiveness of current antidepressant treatments are still poorly understood. Despite this lack of knowledge, some efficient pharmacologic and nonpharmacologic treatments for depression are available today, but less than 50% of patients show full remission. Antidepressant medications that inhibit reuptake of monoamines or the monoamine oxidase enzyme are effective but involve side effects that cannot be tolerated by many patients. Only a small conceptual advance in antidepressant treatments has occurred since the 1950s; however, current medications produce fewer side effects (3,4). Still today, one of the most effective treatments known for depression is electroconvulsive therapy (ECT) (4), but this treatment is mainly used in patients with severe forms of depression due to its profound side effects. Obviously, understanding the neuronal mechanisms and neurochemical factors underlying depression and effective antidepressant treatments, such as ECT, is necessary for development of better treatments.

Brain-derived neurotrophic factor (BDNF) was found to be critically involved in depressive behavior and suicide events (e.g., (5–10)) and in the effects of antidepressant treatments (7,11). Interestingly, opposing roles were reported for BDNF in the hippocampus and the ventral tegmental area (VTA) (12). Brain-derived neurotrophic factor overexpression (OE) within the dorsal dentate gyrus (dDG) (the main hippocampal input) of adult rats blocks the behavioral effects of chronic mild stress (9), a widely used paradigm that induces depressive-like behavior and reduces hippocampal BDNF expression (13–16). Moreover, we have recently shown that BDNF knockdown (KD) in the rat dDG (but not the cornu ammonis 3) is sufficient to induce depressive-like behaviors (10). In addition, hippocampal BDNF level was shown to increase following ECT and administration of various antidepressant medications (11,17–19). Furthermore, infusion of BDNF into rat hippocampus results in antidepressant-like effects (20), and BDNF elevation within mice dentate gyrus (DG) is necessary for the action of the antidepressant drugs desipramine and citalopram (11). On the other hand, BDNF ablation from the VTA of mice induces an antidepressant-like effect in the social defeat stress paradigm (21) and blockade of BDNF action in the nucleus accumbens (NAC) by overexpressing the dominant negative mutant of tyrosine kinase receptor B (the BDNF high-affinity receptor) also induces antidepressant-like effect (22). This emphasizes the role of VTA BDNF in depressive behavior, as VTA is thought to be a major source of BDNF protein to the NAC (23). Moreover, infusion of BDNF into the rat VTA produces depressive-like behavior (22). The robust antidepressant-like effect of ECT has led us to explore whether altered hippocampal or VTA BDNF expression has a causal role in this action.

**Methods and Materials**

**High Titer Lentiviral Preparation**

Lentiviral vectors (LVs) were prepared as we previously described (9,10). To induce BDNF knockdown, we used our previously...
described LV (10), which expresses short hairpin RNA complementary to the rat BDNF coding sequence (LV-shBDNF). Lentiviral vectors expressing scrambled short hairpin RNA sequence were used as control vectors. To overexpress BDNF, we used our previously described LV (9), which expresses the rat BDNF coding sequence (LV-BDNF), and the LV expressing green fluorescent protein only as control vector.

**Animals**

Ten-week-old male Sprague Dawley rats (Harlan Laboratories, Rehovot, Israel) were housed individually on a standard 12-hour light/dark schedule (lights on at 7:00 AM) within an animal housing facility located at the Weizmann Institute of Science. Food and water were available ad libitum. All animals were handled according to the regulations formulated by the Weizmann Institute of Science Institutional Animal Care and Use Committee, which are in complete accordance with the National Institutes of Health guidelines for care and use of laboratory animals.

**Intracranial Surgery**

Rats were anesthetized with ketamine (170 mg/kg) and acepromazine (1.7 mg/kg) and placed in a stereotaxic frame. Stainless steel guide cannulae (Plastics One, Roanoke, Virginia) were implanted bilaterally into the dDG of adult rats, using the coordinates we have previously described (9,10): −3.8 anteroposterior, +2.4 mediolateral, and −1.95 dorsoventral (1.5 mm above the injection site) at a lateral angle of 10°. In separate groups of rats, stainless steel guide cannulae were implanted bilaterally into the VTA, using the following coordinates: −6.1 anteroposterior, +1.85 mediolateral, and −6.83 dorsoventral (1.5 mm above the injection site) at a lateral angle of 10°. All coordinates are relative to bregma and according to the atlas of Paxinos and Watson (24).

**Microinjection of LVs**

Lentiviral vector infections were performed as we have previously described (9,10). In brief, bilateral microinjections (2 µL over 4 minutes) of either LV-shBDNF, LV expressing scrambled short hairpin RNA sequence, LV-BDNF, or LV expressing green fluorescent protein were performed into the aforementioned coordinates using a dual channel MAB 40 microdialysis pump (Microbiotech/SE AB, Stockholm, Sweden) and an injection needle extending 1.5 mm below the edge of the guide cannulae. To increase the effectiveness of the infections, microinjections were repeated three times every other day.

**Antidepressant Treatments**

A week following the first LV infection, rats were chronically treated with ECT (100 V, 50 Hz, 1.5 seconds) once a day for 10 days using Siemens Konvulsator 2077 S (Siemens, Malvern, Pennsylvania) via earclip electrodes, as we have previously described (25). Rats were mildly anesthetized using carbon dioxide (9,26,27), and stimulation parameters were set to achieve a tonic-clonic seizure lasting at least 10 seconds. The sham-treated rats experienced the same procedure but without delivering the electric current.

**Sucrose Preference**

Sucrose preference measures were performed as previously described (9,10,16). Following ECT, rats had access at their home cage to two bottles located side by side, which contained either tap water or 2% sucrose solution. Fluid consumption was recorded by weighing the bottles every morning between 9:30 AM and 10:00 AM. Rats’ fluid consumption was tested for 4 days, followed by 1 day of tap water only, and then bottle positions were switched and rats were tested for an additional 4 days to control for side preference. Sucrose preference for each rat was defined as the average ratio between sucrose to water consumption for the whole period excluding the first measure at each side.
Forced Swim Test

A modified forced swim test (FST) was conducted in a cylindrical tank (40 cm high and 18 cm in diameter; constructed at the Weizmann Institute), as we previously described (9,10,16,28). The water temperature was kept at 25°C (2°C above room temperature) and the water level was such that the rat could not touch the bottom with its hind paws. Rats were exposed to the swim tank for 10 minutes on the first day and 5 minutes on the second day and were videotaped. Video films of the second day of each FST session were analyzed by a software developed in our laboratory, which continuously examines movement of pixels adjacent to the rat limbs and detects fine alterations in mobility throughout the test, providing an automated score that presents the total movement of limbs during the test (28,29). In addition, immobility time was measured manually, using a stopwatch, by a trained observer blinded to the experimental groups. The average score for each group was calculated; results are presented as a percentage relative to the average score of the sham-treated control LV group, defined as 100%.

In vivo BDNF Enzyme-Linked Immunosorbent Assay

Brain-derived neurotrophic factor protein levels were measured by enzyme-linked immunosorbent assay, as we have previously described (9,10). Rats were decapitated 7 days after the FST and their brains were extracted, immediately frozen in isopropanol on dry ice, and stored at −80°C. Bilateral tissue punches were obtained from coronal sections used for punches of the prefrontal cortex (PLC), NAc, amygdala (Amg), dDG, and VTA were taken anteroposterior relative to bregma from 4.7 to 2.7, 2.7 to −7, −2 to −3, −3 to −5, and −5 to −6, respectively (Figure S1 in Supplement 1). Protein extraction and sandwich enzyme-linked immunosorbent assay were performed. Brain-derived neurotrophic factor concentration was normalized per total protein or per tissue weight. Similar results were obtained when normalized in both ways. Results are presented after normalization to total protein levels as previously described (9,10,16).

Statistical Analysis

Results are expressed as mean ± SEM. Analyses of sucrose preference, FST activity, and BDNF levels were performed using two-way analysis of variance (ANOVA) (LV × ECT), followed by post hoc Student t tests, except for the experiment measuring the effects of VTA BDNF KD, which included only two groups and was analyzed just by the Student t test.

Results

Increased Hippocampal BDNF Expression Is Not Essential for the Antidepressant-like Effect of ECT

Microinjections of the LV-shBDNF or the control LV were performed before the ECT or sham treatments Figure 1A. Two-way ANOVA revealed significant main effects of both BDNF KD [F(1,50) = 40.58, p < .001] and ECT [F(1,50) = 9.238, p < .01] on dDG BDNF expression levels (Figure 1B). Post hoc analysis showed that ECT increased BDNF expression in the dDG of the control LV treated rats (Figure 1B; p < .05,
relative to the sham-treated group infected with the control LV). Brain-derived neurotrophic factor KD reduced BDNF expression levels in the dDG of sham-treated rats, as well as in the dDG of the ECT treated rats (Figure 1B; $p < .05$ relative to the control LV sham group). Analysis of the activity during the FST revealed significant main effects of both BDNF KD [$F(1,48) = 4.965, p < .05$] and ECT [$F(1,48) = 13.903, p < .001$]. Electroconvulsive therapy increased the activity of the control LV rats during the FST (Figure 1C; $p < .01$, relative to the control LV sham group), while dDG BDNF KD reduced the activity measured in sham-treated rats (Figure 1C; $p < .05$). Importantly, while the antidepressant-like effect of desipramine and citalopram in the FST are blocked by DG BDNF KD (7,11), the antidepressant-like effect of ECT was not blocked by dDG BDNF KD, as the FST scores in this group were not significantly reduced and even tended to increase relative to the control LV sham group (Figure 1C). Similar results were observed when analyzing the immobility time of these rats (Figure S2 in Supplement 1). Analysis of sucrose preference revealed a significant main effect of BDNF KD [$F(1,50) = 7.902, p < .01$]. While BDNF KD significantly reduced the sucrose preference of sham-treated rats ($p < .05$), ECT blocked this effect and normalized sucrose preference levels (Figure 1D; Figure S3 in Supplement 1).

**Hippocampal BDNF KD and ECT Alter BDNF Expression in the Brain Reward System**

The effect of ECT and dDG BDNF KD on BDNF expression were measured also in other reward-related brain regions (Figure 2). Electroconvulsive therapy induced main effects on BDNF expression in the VTA [$F(1,35) = 8.051, p < .01$] and the post hoc analysis revealed that VTA BDNF expression was significantly reduced regardless of whether rats were or were not subjected to dDG BDNF KD (Figure 2D; $p < .05$, relative to the control LV sham group). On the other hand, no effects of ECT on BDNF expression were detected in the PLC, NAc, or Amg (Figure 2). In addition, the BDNF KD in the dDG induced main effects on BDNF expression in the PLC [$F(1,33) = 5.099, p < .05$] and Amg [$F(1,35) = 10.619, p < .01$] but not on the VTA or NAc. Post hoc analysis revealed that dDG BDNF KD by itself was sufficient to induce a significant increase of BDNF expression in the PLC and Amg of sham-treated rats (Figure 2A, C; $p < .05$).

**BDNF KD in the VTA Induces Antidepressant-like Effects**

As ECT significantly reduced VTA BDNF expression (Figure 2D) and caused antidepressant-like effects, even after KD BDNF in the dDG (Figure 1C, D), we were curious to test whether reduction in VTA BDNF expression by itself can induce antidepressant-like effects. Reduction in BDNF expression within the mice VTA was previously shown to induce an antidepressant-like effect at the social defeat stress paradigm (21). Therefore, in the next experiment, we induced BDNF KD in the VTA and measured behavioral outcomes and BDNF expression in reward-related regions Figure 3A. Indeed, while microinjection of the LV-shBDNF into the VTA effectively reduced local expression of BDNF (Figure 3B; $p < .05$), activity in the FST was significantly increased (Figure 3C; $p < .05$), and the immobility time was significantly decreased (Figure 4A; $p < .05$). Furthermore, VTA BDNF KD induced a significant increase in sucrose preference (Figure 3D; Figure S5 in Supplement 1; $p < .05$). The VTA BDNF KD did not induce significant alterations in BDNF expression within the PLC, NAc, Amg, or dDG (Figure 4A–D).

**Reduction in VTA BDNF Expression Is Necessary for the Antidepressant-like Effects of ECT**

To test whether the reduction in BDNF expression within the VTA is necessary for antidepressant-like effects of ECT, we microinjected the LV-BDNF into the VTA, which induces local overexpression of BDNF (9), and tested whether such manipulation would change the effects of ECT on activity in the FST and on sucrose preference. (A) Lentiviral vector (LV) expressing short hairpin RNA complementary to the rat BDNF coding sequence was microinjected into the VTA of rats and behavioral measurements were performed as presented at the experimental timeline. (B) Brain-derived neurotrophic factor expression within the VTA was measured by enzyme-linked immunosorbent assay to validate the efficiency of the BDNF KD. Activity during the FST (C) and sucrose preference (D) were measured, and results were compared with those of the group infected with the control LV. Values are mean ± SEM (n = 8–9 per group; *p < .05).
significant reduction in VTA BDNF expression of the control LV treated rats (Figure 5B; \(p < 0.05\)), but the groups infected with LV-BDNF before treatment with ECT displayed almost normal levels of VTA BDNF expression (Figure 5B). In the FST, main effects of ECT \([F(1,38) = 4.82, p < 0.05]\) and BDNF OE \([F(1,38) = 7.37, p < 0.01]\), as well as ECT \(\times\) BDNF OE interaction \([F(1,38) = 4.34, p < 0.05]\), were observed. Post hoc analysis revealed that ECT increased the activity of the control LV rats at the FST (Figure 5C; \(p < 0.05\)); however, this antidepressant-like effect of ECT was blocked by VTA BDNF OE (Figure 5C). Similar results were observed when analyzing the immobility time of these rats (Figure S6 in Supplement 1). In the sucrose preference test, no effect of ECT was detected in control LV rats; however, a main effect of VTA BDNF OE was observed \([F(1,38) = 4.13, p < 0.05]\). Post hoc analysis revealed that while VTA BDNF OE significantly decreased sucrose preference in sham-treated rats (Figure 5D; Figure S7 in Supplement 1; \(p < 0.05\)), ECT blocked this effect of BDNF OE (Figure 5D; Figure S7 in Supplement 1).

**VTA BDNF OE and ECT Alter BDNF Expression in Reward-Related Brain Regions**

A main effect on BDNF expression within the PLC was observed following VTA BDNF OE \([F(1,27) = 21.41, p < 0.001]\). Post hoc analysis showed that BDNF expression was significantly decreased at the PLC of rats that were subjected to VTA BDNF OE and treated with either sham or ECT (Figure 6A; \(p < .01\)). In addition, main effect of ECT on dDG BDNF expression was observed \([F(1,33) = 10.24, p < .01]\). Post hoc analysis revealed that BDNF expression was increased significantly in the dDG of ECT treated rats, in both the BDNF OE and control LV groups (Figure 6D; \(p < .05\)). Finally, ECT or VTA BDNF OE did not induce alterations in BDNF expression in the NAc or Amg (Figure 6B, C).

**Discussion**

In the present study, we identified a critical effect of ECT on expression of BDNF in the VTA, which was necessary for its antidepressant-like effect. Ventral tegmental area BDNF OE was sufficient to block the effect of ECT, and the behavioral significance of the attenuation in VTA BDNF expression induced by ECT was further supported by the finding that VTA BDNF KD alone induced an antidepressant-like effect. In addition, BDNF KD within the mice VTA was shown to have an antidepressant-like effect in the social defeat stress paradigm (21). On the other hand, we did not find such a critical role for dDG BDNF expression in the antidepressant-like effect of ECT, in contrast to studies on desipramine and citalopram (7,11), which showed an essential role of hippocampal BDNF expression in behavioral effects of these antidepressant medications.

Interestingly, while BDNF KD in the hippocampus induces depressive-like behavior (10) and BDNF OE in the hippocampus induces an antidepressant-like effect (9), in this study, we show that BDNF KD in the VTA induces an antidepressant-like effect; see also (21). These region-dependent opposite effects of BDNF may be mediated by alterations in cyclic adenosine monophosphate response element binding protein (CREB), which is one of the BDNF effector proteins (30). Indeed, CREB OE within the rat NAc (which receives a major source of BDNF protein from the VTA) causes
Pressants increase CREB activity within this region (6,34). Moreover, an important mediator of antidepressant-like effects, as many antidepressants increase CREB activity within this region (6,34). In the hippocampus, however, CREB seems to be an important mechanism of antidepressant-like effects, as many antidepressants increase CREB activity within this region (6,34). Moreover, CREB OE within rats' DG produces antidepressant-like effects (35). Therefore, the increase in hippocampal BDNF expression that is necessary for the antidepressant-like effect of desipramine and citalopram (7,11) may relate to activation of hippocampal CREB, while the reduction of VTA BDNF expression that we found to be necessary for the antidepressant-like effect of ECT may relate to reduction in CREB activity in VTA terminals within the NAc.

Although this study was designed to evaluate whether local changes in BDNF expression are essential for an antidepressant-like effect of ECT, our measurements of BDNF levels in the sham-treated groups revealed interactions between BDNF expression in different brain regions. For example, hippocampal BDNF KD induced elevations in PLC BDNF expression but did not alter VTA BDNF expression. On the other hand, VTA BDNF OE caused reduction in PLC BDNF expression without affecting hippocampal BDNF expression in sham-treated rats. These regional interactions may relate to the important role of BDNF in synaptic plasticity (36) and the reported functional connectivity between these regions. Indeed, electrophysiological studies have characterized a sensitive balance between PLC and hippocampal inputs to the NAc that is mediated by the dopaminergic system (37). Therefore, hippocampal BDNF KD in the present study can enhance neuroplasticity (and BDNF expression) in the PLC projections, and VTA BDNF OE may suppress plasticity in PLC projections.

It is interesting to note that while ECT caused an antidepressant-like effect at the FST and this effect was blocked by VTA BDNF OE, we did not observe a direct effect of ECT on sucrose preference. On the other hand, BDNF OE within the VTA significantly reduced sucrose preference (which is interpreted as an anhedonic-like effect) and this effect was blocked by ECT, but the BDNF OE did not affect activity in the FST. We suggest that this difference between the behavioral outcomes of ECT measured in the sucrose preference and the FST relate to the specific change in BDNF expression in the VTA, relative to the normal (control) BDNF expression levels. While ECT significantly decreased VTA BDNF expression levels and the LV-BDNF (BDNF OE) increased VTA BDNF expression levels, the combined effect of these two manipulations was normalization of BDNF expression levels (Figure 5B). These normal VTA BDNF expression levels (following BDNF OE and ECT) blocked the antidepressant-like effect of ECT at the FST and the anhedonic-like effect of VTA BDNF OE at the sucrose preference. Interestingly, at the same time that ECT reduces VTA BDNF expression levels, it increases BDNF expression in the dDG, but this increase in dDG BDNF levels is not sufficient to rescue the effect of ECT following VTA BDNF OE. Therefore, VTA BDNF alterations seem to have more powerful effects on depressive-like behavior than those of BDNF alterations in the dDG. Further investigation of the role of VTA BDNF expression and downstream mechanisms in depressive-like behavior and antidepressant mechanisms should be tested in future studies using additional animal models and behavioral measures.

In conclusion, while the antidepressant mechanism of desipramine and citalopram depends on elevation of hippocampal BDNF expression, in this study we show that the antidepressant mechanism of ECT depends on reduction of VTA BDNF expression. These findings highlight the differential roles of BDNF in the hippocampus and the VTA with regard to depression and responsiveness to antidepressant manipulations. Given that ECT is one of the most effective treatments known for depression today that can induce beneficial effects even in drug-resistant patients, these differential effects of ECT and antidepressant medications on local BDNF expression levels may explain basic characteristics of drug-resistant patients. For example, if desipramine or citalopram would not induce elevation in dDG BDNF expression in these patients (e.g., due to genetic alterations or inflammation), ECT may still induce an anhedonic-like behavior, as indicated by the reduced rewarding effects of sucrose, cocaine, and morphine (2,31,32). In addition, elevation of CREB within the NAc produces depressive-like behavior (32,33). In the hippocampus, however, CREB seems to be an important mediator of antidepressant-like effects, as many antidepressants increase CREB activity within this region (6,34). Moreover, BDNF OE. Activity of the rats during the FST (C) and sucrose preference (D) were measured, and results are expressed relative to the average score measured in the sham treated control LV group. Values are mean ± SEM (n = 10–11 per group; *p < .05).

Figure 5. The effects of ventral tegmental area (VTA) brain-derived neurotrophic factor (BDNF) overexpression (OE) and electroconvulsive therapy (ECT) on BDNF expression, activity during the forced swim test (FST), and sucrose preference. (A) Lentiviral vector (LV) expressing the rat BDNF coding sequence was microinjected into the VTA of rats to induce local BDNF OE and then the rats were subjected to ECT or sham treatments, followed by behavioral measurements as presented at the experimental timeline. (B) BDNF expression levels were measured to test for differences induced by the ECT and to validate the efficiency of the BDNF OE. Activity of the rats during the FST (C) and sucrose preference (D) were measured, and results are expressed relative to the average score measured in the sham treated control LV group. Values are mean ± SEM (n = 10–11 per group; *p < .05).

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effect by reduction of BDNF levels in the VTA. Therefore, evaluation of BDNF genetic differences may increase our ability to provide the most appropriate treatment to an individual patient and maximize the antidepressant effect of each treatment to a certain individual. Future antidepressant treatments should address the opposite effects of BDNF in the hippocampus and VTA and seek ways to specifically alter BDNF within these brain regions to optimize and facilitate antidepressant outcomes with minimal side effects.

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Supplementary material cited in this article is available online.


