Site-Specific Antidepressant Effects of Repeated Subconvulsive Electrical Stimulation: Potential Role of Brain-Derived Neurotrophic Factor

Roman Gersner, Erika Toth, Moshe Isserles, and Abraham Zangen

Background: Electroconvulsive therapy (ECT) is a very effective treatment for major depression. This method involves robust nonfocal stimulation of the brain and can normalize both neurochemical alterations and depressive behavior in animal models. We hypothesized that short stimulation sessions of specific reward-related brain sites might induce similar effects.

Methods: In the present study we compared behavioral and neurochemical effects produced by ECT and by repeated stimulation of reward-related brain sites, in a widely used rat model for depressive behavior induced by chronic mild stress (CMS). Different groups of rats received 10 sessions of either electroconvulsive shocks or subconvulsive electrical stimulation (SCES) of specific brain sites with an implanted electrode. The SCES temporal parameters were similar to those used in transcranial magnetic stimulation studies in humans. A battery of behavioral tests and measurements of brain-derived neurotrophic factor (BDNF) levels were used to assess the effectiveness of these treatments relative to sham treatments.

Results: Repeated SCES of either the nucleus accumbens (NAC) or the ventral but not the dorsal prelimbic cortex (PLC) reversed the main behavioral deficit and the reduction of BDNF levels in the hippocampus that were induced by CMS. The ECT was more effective because it also normalized a behavioral deficit associated with anxiety but produced a learning and memory impairment.

Conclusions: This study implicates the ventral PLC and the NAC in the pathophysiology of depressive behavior and suggests that local intermittent SCES can induce an antidepressant effect similar to that of ECT, without the cognitive impairment caused by the convulsive treatment.

Key Words: Brain-derived neurotrophic factor, chronic mild stress, depression, dorsal hippocampus, ECT, subconvulsive electrical stimulation

Major depression is the leading source of disability in the Western World, with a lifetime prevalence of up to 16% (1). Because one-fourth of all patients fail to respond to adequate antidepressant pharmacotherapy (2), additional treatment options are needed. Electroconvulsive therapy (ECT) is considered a very effective antidepressant treatment but necessitates administering general anesthesia, induces a seizure, and causes significant memory (3,4) and learning (5) impairments.

It is proposed that targeting specific brain circuits for stimulation can achieve a therapeutic effect that is as good as the effect caused by a broader stimulus while minimizing the side effects. Over the past decade, several novel neurostimulation modalities have been in development (6). Transcranial magnetic stimulation (TMS), a nonconvulsive and nonsurgical stimulation modality, has proven some antidepressant efficacy in most clinical trials, usually with modest clinical effects. Possible explanations for the modest effect are suboptimal stimulation parameters, short treatment durations, and poor target areas (7).

The prefrontal cortex (PFC), nucleus accumbens (NAC), ventral tegmental area (VTA), hippocampus, and other limbic structures are thought to be involved in reward and affective circuitry, thus being potential target areas for subconvulsive stimulation therapy in depression (8). These structures, however, reside deep in the brain, where direct stimulation is not feasible with standard TMS coils (9). We have, however, developed a coil that allows direct stimulation of deeper brain regions (producing effective stimulation of both superficial and deeper areas) and tested its safety and efficacy when used over the prefrontal or motor cortices (9–12).

A different nonpharmacological approach for the treatment of depression is chronic ongoing (rather than daily short sessions) high-frequency deep brain stimulation (DBS) with an implanted electrode. The first study involved 6-month stimulation of white matter tracts adjacent to the subgenual cingulate gyrus. In that study, four of six patients had improved immediately and remained in response by the end of the 6-month period (13). Since then, several more studies reported antidepressant effects of DBS also in the nucleus accumbens (e.g., 14,15), anterior capsule (16), and inferior thalamic peduncle (17). Although this method involves a surgery and localized chronic stimulation, the TMS approach is conceptually more similar to ECT, because it involves repeated short sessions of stimulation that are thought to produce long-lasting effects, beyond the minutes of stimulation each day.

In the present study, we compared behavioral and neurochemical effects induced by 10 electroconvulsive sessions with those induced by 10 short sessions of focal subconvulsive electrical stimulation (SCES) applied to reward-related brain regions with an implanted electrode. We used an animal model that exhibits depressive-like behavior and neurochemical alterations induced by chronic mild stress (CMS) (18,19). It has been suggested that the corticostriatal interaction plays a role in the antidepressant mechanism of ECT (20). Consequently, we tested the effectiveness of SCES treatment in the prelimbic cortex (PLC) and NAC, which are homologous to human ventromedial PFC

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Received Mar 16, 2009; revised Sep 13, 2009; accepted Sep 14, 2009.

0006-3223/10/$36.00
doi:10.1016/j.biopsych.2009.09.015

(21) and ventral striatum (22), respectively. We applied focal brain stimulation with an implanted electrode as in a DBS-like setup, whereas stimulation pattern (frequency, session length, train duration, and intertrain interval) were similar to those used in rTMS-dorsolateral PFC studies in humans (23).

Chronic mild stress is a widely used rodent model that mimics some symptoms of depression in humans, especially anhedonia. In this model, rats are exposed to a series of chronic mild and unpredictable environmental stressors, resulting in reduction of preference for sweetened solutions and sexual behavior, decreased response to rewarding electrical brain stimulation, and decreased exploration of novel environments (19,24,25). Furthermore, this model mimics some neurochemical alterations induced in depression (26), including reduction in hippocampal neuroplasticity (19). Converging lines of evidence point to a critical role of brain-derived neurotrophic factor (BDNF) in neuroplasticity and depression (27,28). Reduced BDNF levels are found in depressive subjects, and different types of stress cause a reduction in hippocampal BDNF levels, whereas antidepressant drugs and ECT increase the expression of BDNF in the hippocampus, striatum, and frontal cortex (19,29–31). Moreover, hippocampal BDNF is necessary for behavioral effects of antidepressants in a mouse model (32). In the present study, BDNF levels were measured after CMS, followed by convulsive or nonconvulsive brain stimulation treatments.

Methods and Materials

Experimental Design

The experimental design is represented in Figure 1. Animals intended for the SCES experiments (real or sham) underwent surgery to implant electrodes before the initiation of the CMS protocol. After completion of the 4-week CMS protocol, either ECT or SCES treatment (active or sham; n = 10–14/group) was performed for 10 days. Subsequently, animals underwent behavioral tests during a 3-week period in the following sequence: sucrose preference, home-cage locomotion, exploration, forced swim test, and Morris water maze. In some groups of rats, brains were removed and punches of specific brain sites were taken for BDNF enzyme-linked immunosorbent assay (ELISA) measurements.

Animals

Male Sprague-Dawley rats (60 days old at experiment initiation) were singly housed and maintained under a 12 hour/12 hour light-dark cycle with food and water ad libitum. All animal experiments were conducted according to the Institutional Animal Care and Use Committee (IACUC) guidelines.

Surgery

Rats were anesthetized intraperitoneally with a combination of ketamine hydrogen chloride (170 mg/kg) and acepromazine maleate (1.7 mg/kg). For PLC stimulation, to reflect on human TMS studies (e.g., 33), electrodes were implanted unilaterally into the left hemisphere (coordinates [mm]: +3.7 anteroposterior [AP], −.4 mediolateral [ML], 3.5 dorsoventral [DV]; Figure S1A in Supplement 1) (34). Initial results followed by histological analysis revealed different behavioral effects between dorsal and ventral PLC (vPLC) placements, therefore placements in the following groups were directed into the dorsal (DV = 3.0) or ventral (DV = 4.0) PLC (as shown by dark and bright gray, respectively, in Figure S1A in Supplement 1). For NAC stimulation, left unilateral electrodes were implanted at +1.6 AP, −1.0 ML and 7.0 DV (Figure S1B in Supplement 1). Animals were allowed 1 week for recovery before initiation of the CMS procedure.

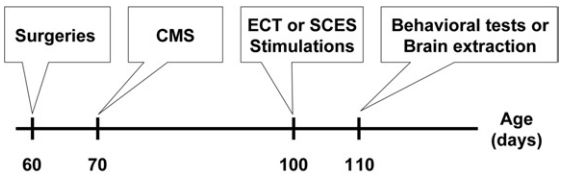

Figure 1. Experimental design. The time schedule represents different procedures according to the animals' age. CMS, chronic mild stress; ECT, electroconvulsive therapy; SCES, subconvulsive electrical stimulation.

CMS Procedure

The CMS procedure (18) has been adopted with modifications as previously reported (19) and detailed in Supplement 1.

Electroconvulsive Shock

Electroconvulsive shock (ECS) (100 V, 50 Hz, 1.5 sec) was administered once a day for 10 days with Siemens Konvulsator 2077 S (Siemens, Malvern, Pennsylvania) via ear-clip electrodes. Stimulation parameters were set to achieve a tonic-clonic seizure lasting at least 10 sec. The ECS treatment was given with mild anesthesia (ketamine 85 mg/kg, and promace .85 mg/kg) according to the local IACUC requirements.

SCES

A 10-min-long SCES session was administered every day for 10 days. Rats were connected to an electrical stimulator via a flexible wire and placed in their home-cage. Although stimulation was delivered focally by an implanted electrode as in DBS, the stimulation pattern, length (10 min/day), and temporal parameters were chosen to imitate TMS treatment of depression in humans. Each stimulation cycle consisted of 100 pulses during 5 sec (20 Hz) followed by a 20-sec pause. Pulse width was .2 msec with intensity of 400 μA (an intensity that was previously used effectively and did not seem to disturb normal animals' behavior) (35). Sham control groups were treated similarly without applying any current. Non-contingent stimulation might be aversive (as suggested in studies using much higher stimulation frequencies); however, the rats did not seem to develop resistance or avoidance to our treatment. In addition, none of the rats experienced a seizure during or after the SCES treatment.

Sucrose Preference Test

The sucrose preference test was performed as previously described (19) and detailed in Supplement 1.

Home-Cage Locomotion

Continuous monitoring of locomotion was performed in the home cages, with an automated system (Inframot, TSE, Bad Homburg, Germany), on the basis of infrared sensors located above each rat's home cage, as described previously (19). The night locomotion (8:00 PM–8:00 AM) score was analyzed for each rat for 5 consecutive nights.

Exploration and Novelty-Induced Behavior

Rats were exposed to a novel environment for 10 min, and their activity was measured with an automated system (Actimot, TSE) as described previously (19). Horizontal and vertical activities and the number of entries into the center of the exploration box were analyzed as detailed in Supplement 1.

Swim Test

A modified forced swim test was conducted in a cylindrical tank as described previously (36) and detailed in Supplement 1.

Morris Water Maze

The Morris water maze was set to study spatial reference memory and learning as previously described (37) with modifications, as detailed in Supplement 1.

Histology and ELISA

After brain extraction, the electrode implantation region was taken for Nissel staining and histological analysis of electrode placement. In some groups of rats, bilateral tissue punches of NAC, striatum, VTA, and dorsal and ventral hippocampus were extracted from approximately 1.5 mm coronal sections. Protein extraction and sandwich ELISA were performed as described previously (38) and detailed in Supplement 1.

Statistical Analysis

Results are presented as means ± SEM. Significance of treatment effect was analyzed by one-way analysis of variance (ANOVA) followed by Dunnet's post hoc test with CMS sham as the reference group. Differences between groups in the learning process of the Morris water maze were determined by repeated-measures ANOVA. The statistical analysis of the ECT (no surgery) and the SCES (surgery) experimental groups was separated. The correlations between depth of electrode placement, sucrose preference, and BDNF levels were analyzed by Spearman's Rank Correlation test. All analysis was done with StatView 5.0. A p value $< .05$ was considered statistically significant.

Results

Sucrose Preference

The CMS induced significant reductions in sucrose preference relative to the control groups (Figure 2), as described previously (19). A comparison between sucrose preference scores, measured in the ECT experimental groups revealed a significant group effect [$F(2,38) = 5.28$, $p = .0062$; Figure 2A). Post hoc analysis showed that sucrose preference was significantly decreased in sham-treated CMS animals relative to non-CMS control subjects, whereas ECT significantly increased sucrose preference in the CMS animals (Figure 2A).

A comparison between sucrose preference scores measured in the SCES experimental groups revealed a significant group effect [$F(4,41) = 4.864$, $p = .0026$; Figure 2A]. Post hoc analysis showed that sucrose preference of the sham CMS groups was significantly decreased relative to the sham control group. In animals exposed to CMS, SCES of the vPLC and NAC but not dorsal PLC (dPLC) significantly increased sucrose preference relative to the sham-treated CMS control subjects (Figure 2A). Moreover, a significant correlation was obtained between the depth of the stimulation site and the sucrose preference score [$R^2 = .44$, $F(15) = 11.06$, $p = .0051$; Figure 2B; depth is relative to the PLC midline presented in Figure S1A in Supplement 1].

By contrast, in control animals (not exposed to CMS), SCES of the vPLC or the NAC did not induce significant effects on sucrose preference (Figure S2A in Supplement 1).

Exploration and Novelty-Induced Behavior

The total distance traveled (Figure 3A) and number of rearings (Figure 3B) did not significantly vary from one group to another; however, the number of visits in the center of the arena was affected by CMS (Figure 3C). One-way ANOVA on the number of center visits indicated a significant main effect in the ECT experimental groups [$F(2,27) = 6.953$, $p = .037$; Figure 3C]. Post hoc analysis revealed that animals subjected to CMS visited the central portion of the exploration box significantly less often than control subjects (Figure 3C), indicating increased levels of anxiety. The ECT in CMS animals normalized and significantly increased the number of center visits (Figure 3C).

In the SCES groups, one-way ANOVA revealed an almost significant group effect [$F(4,40) = 2.401$, $p = .0659$; Figure 3C]. The number of center visits of all groups subjected to CMS tended to decrease and were not affected by any of the SCES treatments.

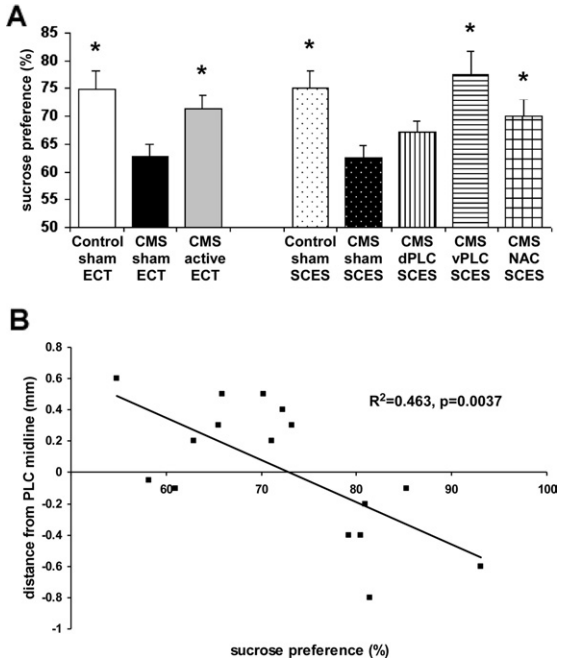

Figure 2. Sucrose preference test. **(A)** Means ± SEMs of the percentage of sucrose (.2%) intake, as calculated from total liquid consumption in the ECT (left) or SCES (right) groups. **(B)** Sucrose preference as a function of electrode depth in the prelimbic cortex relative to the horizontal midline of the prelimbic cortex (PLC) (represented by 0 at the y axis; see Figure S1A in Supplement 1). *$p < .05$, as revealed by Dunnet's post hoc comparisons with the corresponding CMS sham group (ECT or SCES). dPLC, dorsal prelimbic cortex; vPLC, ventral prelimbic cortex; NAC, nucleus accumbens; other abbreviations as in Figure 1.

Home-Cage Locomotion and Forced Swim Test

The CMS protocol did not alter home-cage locomotion (Figure S3A in Supplement 1) and did not change the forced swim test performance (Figure S3B in Supplement 1). Moreover, neither ECT nor SCES treatments affected these behaviors.

Morris Water Maze

In the ECT groups, repeated-measures ANOVA with group as a between-subjects factor and days as a within-subjects factor revealed a significant main effect of group [$F(2,20) = 3.458$, $p = .05$] and days [$F(3,60) = 52.429$, $p < .0001$] and no interaction between the two factors (Figure 4A). Post hoc analysis did not show differences between CMS and control animals but revealed that the escape time of ECT animals was significantly increased relative to the sham CMS group (Figure 4A). In the SCES groups repeated-measures ANOVA did not reveal signif-

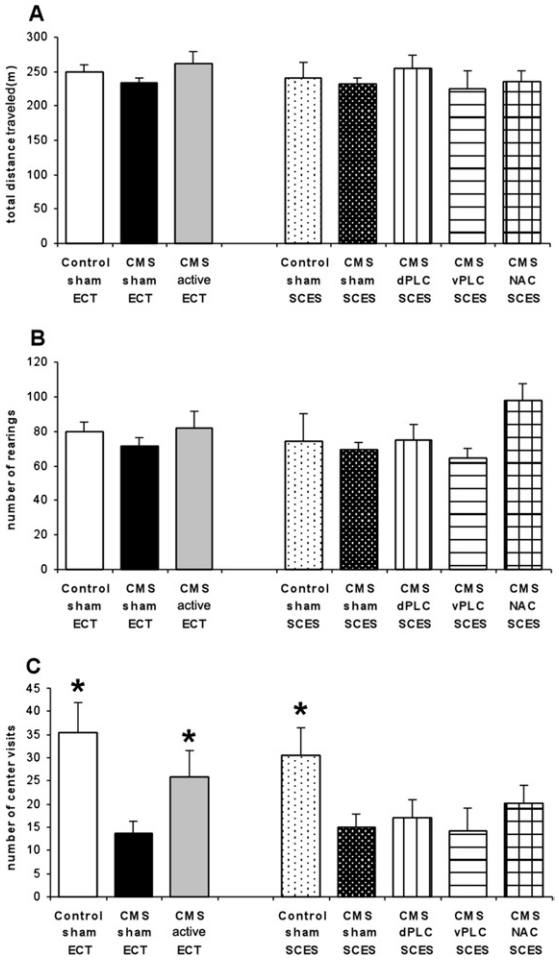

Figure 3. Exploration of a novel environment. The effect of ECT (left) and SCES (right) after CMS on exploratory behavior was automatically measured in computerized exploration boxes. **(A)** Total distance moved; **(B)** total number of rearings; and **(C)** total number of visits in the central portion of the exploration arena during a 10-min test. *$p < .05$, as revealed by Dunnet's post hoc comparisons with the CMS sham ECT group. Abbreviations as in Figures 1 and 2.

icant differences in the learning performance of the Morris water maze (Figure 4B).

On the fourth day, the escape platform was removed, and a probe test was performed. One-way ANOVA indicated a significant main effect in the ECT experimental groups [$F(2,12) = 6.128$, $p = .0147$; Figure 4C]. Post hoc analysis revealed that the active ECT CMS group spent significantly less time in the platform-associated quadrant relative to that of the sham ECT CMS group (Figure 4C). By contrast, SCES treatment of any of the tested regions did not induce behavioral impairments in the Morris water maze (Figure 4C).

Regional BDNF Levels

The BDNF levels were significantly affected by CMS and brain stimulation, especially in the dorsal hippocampus. In the ECT groups, one-way ANOVA revealed a significant group effect [$F(2,26) = 3.857$, $p = .0341$; Figure 5A]. Consistent with our previous results (19), CMS induced a reduction in hippocampal BDNF levels relative to control subjects (Figure 5A). The ECT induced a significant increase in hippocampal BDNF levels (Figure 5A). Similarly, after CMS, SCES treatments induced elevations in hippocampal BDNF levels [$F(4,44) = 6.447$, $p = .0004$; Figure 5A]. By contrast, SCES treatment of control non-CMS animals did not induce alterations in hippocampal BDNF levels (Figure S2B in Supplement 1). Dunnet's post hoc test showed that BDNF levels in the dorsal hippocampus of the sham CMS animals were significantly decreased in comparison with the sham control groups (Figure 5A). The SCES of the vPLC or NAC but not of the dPLC significantly increased BDNF levels in the dorsal hippocampus compared with sham-treated animals (Figure 5A). Moreover, the increase in BDNF levels after PLC stimulation significantly correlated with electrode depth within the PLC (Figure S4 in Supplement 1). By contrast, BDNF levels in the ventral portion of the hippocampus were not altered by CMS or any of the treatments (Figure 5B).

In the striatum, one-way ANOVA indicated significant differences between the ECT experimental groups [$F(2,25) = 4.954$, $p = .0154$; Figure 5C]. Post hoc test showed that BDNF levels were significantly decreased by CMS and that ECT significantly elevated striatal BDNF levels (Figure 5C). Similarly, in the SCES experimental groups, one-way ANOVA indicated a significant effect of treatment [$F(4,43) = 5.793$, $p = .0008$; Figure 5C]. Post hoc analysis showed that BDNF level in the sham CMS group was

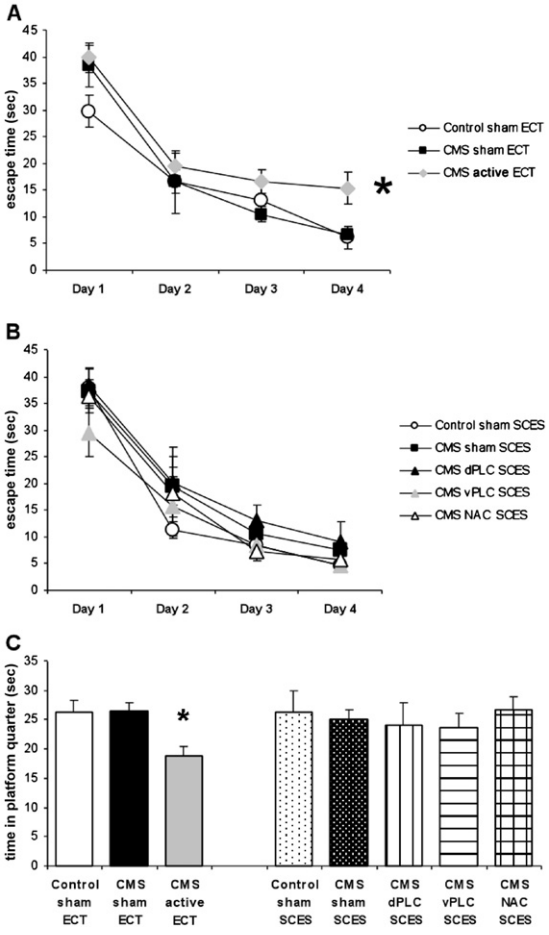

Figure 4. Morris water maze. The effect of ECT or SCES after CMS on learning and memory, as expressed in the Morris water maze, were measured and represented here as means ± SEMs. The learning curve of **(A)** ECT- and **(B)** SCES-treated groups is represented by four daily sessions. **(C)** Probe test performed right after the last training session, to assess short-term spatial memory. *$p < .05$, as revealed by Dunnet's post hoc comparisons with the CMS sham ECT group. Abbreviations as in Figures 1 and 2.

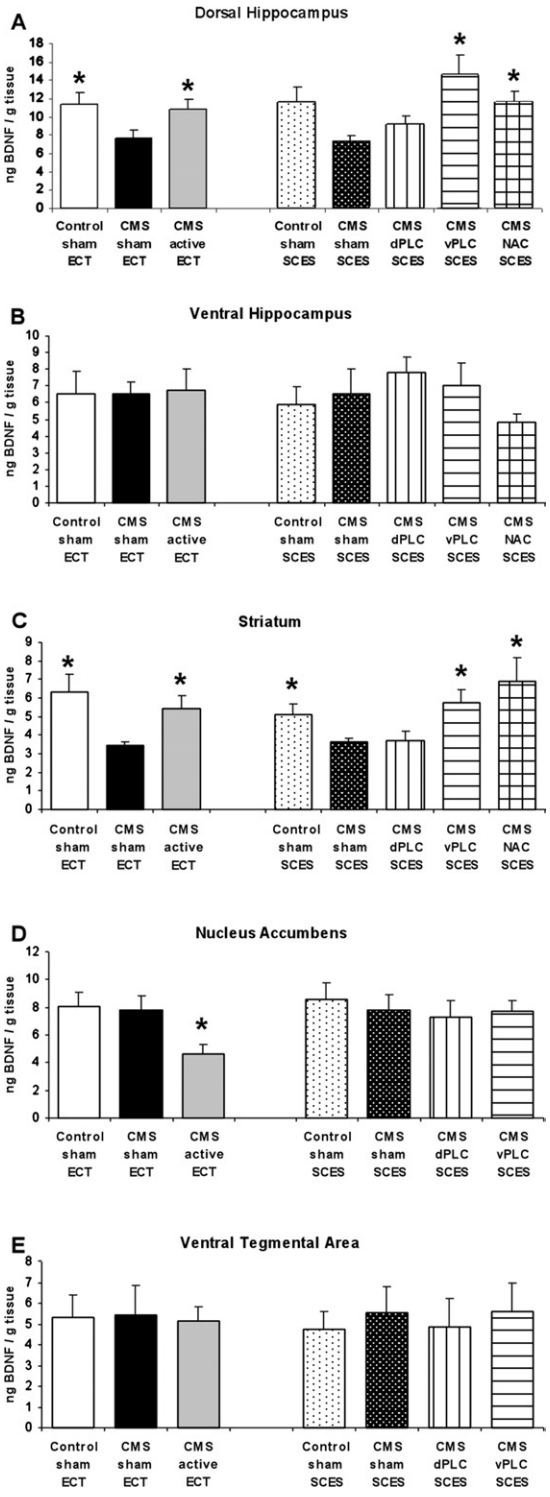

Figure 5. Brain-derived neurotrophic factor (BDNF) levels in specific brain regions. The effects of ECT (left) or SCES (right) after CMS on BDNF levels were measured by enzyme-linked immunosorbent assay. The BDNF levels in the dorsal hippocampus **(A)**, striatum **(B)**, ventral hippocampus **(C)**, nucleus accumbens **(D)**, and ventral tegmental area (VTA) **(E)** are presented as means ± SEMs. For NAC SCES-treated animals, no punches were collected from the NAC or the VTA. *$p < .05$, as revealed by Dunnet's post hoc comparisons with the corresponding CMS sham group (ECT or SCES). Abbreviations as in Figures 1 and 2.

significantly lower than that of the sham control group (Figure 5C). SCES of the vPLC or NAC but not of the dPLC significantly increased BDNF levels in the striatum (Figure 5C). By contrast, SCES treatment of control non-CMS animals did not induce alterations in striatal BDNF levels (Figure S2C in Supplement 1).

In the NAC, one-way ANOVA indicated significant differences between the ECT experimental groups [$F(2,26) = 4.411$, $p = .0224$; Figure 5D]. The CMS had no significant effect on BDNF levels, but ECT induced a significant reduction in BDNF levels in the NAC as indicated by post hoc analysis comparing the active ECT group with the sham CMS group (Figure 5D). In contrast, SCES treatment of the PLC did not affect BDNF levels in the NAC (Figure 5D).

The BDNF levels in the VTA were not altered by CMS or any of the treatments (Figure 5E).

Correlation Between the Behavioral Effect of SCES and BDNF Levels

Because the SCES treatment induced an increase in sucrose preference and in hippocampal and striatal BDNF levels, additional groups of animals were used to study whether these neurochemical and behavioral outcomes correlate. Therefore, after the CMS and the SCES procedures, the same animals were tested for both sucrose preference and BDNF levels (without the stress induced by other behavioral tests after the SCES treatment). In vPLC SCES animals a significant correlation was found between sucrose preference and BDNF levels in the dorsal hippocampus ($R^2 = .38$, $p = .043$; Figure 6A) but not with BDNF levels in the striatum ($R^2 = .26$, p = NS; Figure 6B). By contrast, in NAC SCES animals there was no correlation between sucrose preference and BDNF levels in the dorsal hippocampus ($R^2 = .11$, p = NS, Figure 6C) but a strong correlation with BDNF levels in the striatum ($R^2 = .95$, $p = .035$; Figure 6D).

Discussion

Electroconvulsive therapy is a very effective antidepressant treatment but incorporates major drawbacks, such as the need for general anesthesia, and an accompanied generalized seizure, resulting in undesirable cognitive side-effects. In this study we found that repeated subconvulsive localized stimulation of reward-related brain sites can induce comparable antidepressant and neurochemical effects in an animal model. The impaired sucrose preference induced by CMS, which is considered a measure for anhedonia, was normalized by focal stimulation of the vPLC or the NAC. Electroconvulsive therapy induced a similar therapeutic effect. However, dPLC stimulation did not normalize sucrose preference, and the effectiveness of treatment in the PLC was significantly correlated with the stimulation site depth. Therefore, the antidepressant effect of repeated SCES is site-specific.

The fact that hippocampal and striatal BDNF levels were affected by SCES of the vPLC but not of the dPLC suggests that these site-specific effects relate to the differential connectivity of the dorsal versus the ventral portions of the PLC to subcortical limbic regions (39,40). Indeed, relative to the dorsal portion of the PLC, the ventral portion of the PLC has significantly more projections to subcortical limbic regions (39). For example, the vPLC has more projections to the amygdala (41) that contain excitatory projections to the hippocampus (42) and can lead to increases in BDNF levels (43) and the observed behavioral outcome. Similarly, the NAC might affect the hippocampus indirectly via the lateral hypothalamus or the VTA (44). Stimulation of the NAC can also induce direct effect in the hippocampus

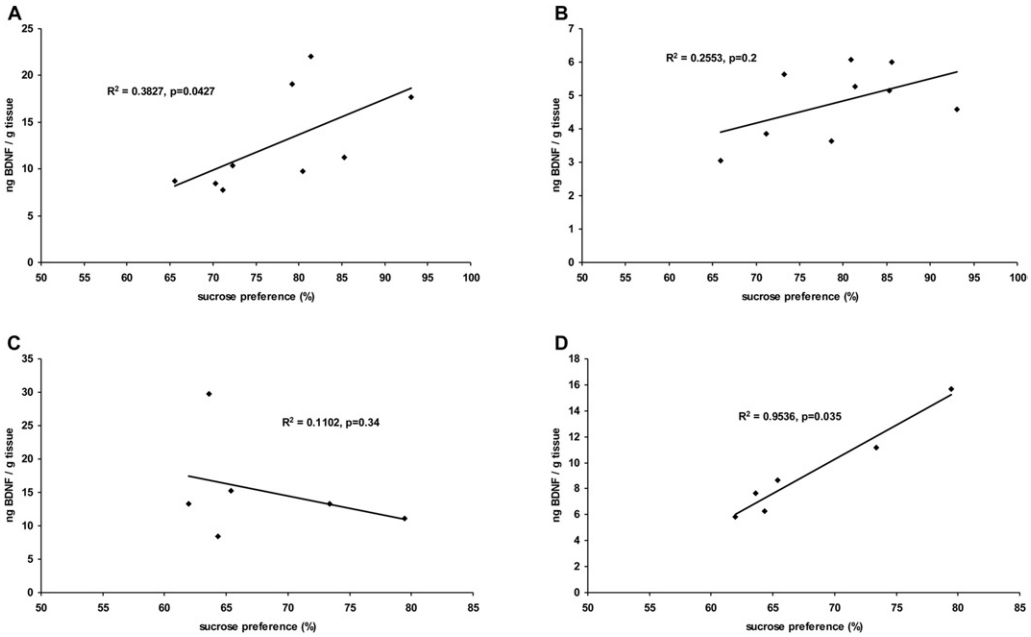

Figure 6. Correlation between sucrose preference and brain-derived neurotrophic factor (BDNF) levels. **(A** and **B)** Sucrose preference as a function of the BDNF levels measured in the dorsal hippocampus **(A)** and the striatum **(B)** of vPLC SCES-treated animals. **(C** and **D)** Sucrose preference as a function of the BDNF levels measured in the dorsal hippocampus **(C)** and the striatum **(D)** of NAC SCES-treated animals. Abbreviations as in Figures 1 and 2.

by antidromic activation of the glutamatergic projections from the hippocampus and subiculum to the NAC (45–47). Activation of these pathways by high-frequency stimulation would activate the hippocampus and induce release of glutamate, and repeated stimulation can enhance neuroplasticity that is associated with increases in BDNF levels (43,48,49). The increases in BDNF levels might be the underlying mechanism of the SCES effects (27,28,32).

Although no therapeutic advantage for ECT over SCES was observed in the sucrose preference tests, only ECT showed effectiveness in the exploration test. The reduced number of center visits, which was the only significant abnormality detected in CMS animals in this test, might reflect elevated anxiety (50,51). The fact that subconvulsive stimulation was not effective in this test is somewhat in line with Hargreaves *et al.*, who failed to prove a TMS anxiolytic-like effect in several rat models for anxiety (52). It is possible that anxiety is mediated by a different brain circuit, affected by the generalized stimulation achieved by ECS but not by focal PLC/NAC stimulation. For example, the present study suggests that these behavioral differences are associated with neuroplasticity induced in specific brain sites as ECT but not SCES, significantly reducing BDNF levels in the nucleus accumbens, whereas both ECT and SCES reversed the effect of CMS on BDNF levels in the dorsal hippocampus and the striatum. Our finding that ECT reduces BDNF levels in the NAC and normalized a measure associated with anxiety seems consistent with previous finding (53) that intra-NAC infusion of a BDNF receptor antagonist results in an antidepressant-like effect in the forced swim test, a test that involves stress and anxiety.

Chronic mild stress did not induce psychomotor retardation or reduced activity in the forced swim test in this study, in line with our previous results (19). In addition, none of the treatment conditions affected these measurements.

It is important to note that use of ketamine during ECT might cause antidepressant effects (54) and is known to induce anticonvulsant effects. However, the control groups received exactly the same dose of ketamine and did not show antidepressant effects. In addition, only a minimal dose of ketamine was used to reduce the adverse effects of the electroconvulsive shocks (as requested by the IACUC). Nevertheless it is possible that the effect of ECT was facilitated by ketamine.

With the Morris water maze, we aimed to evaluate potential cognitive impairments induced by ECT and test the hypothesis that focal stimulation would not cause such learning and memory deficits. The ECT resulted in a significant impairment in both the learning curve and in the time spent on the platform quarter during the probe test. The SCES, by contrast, did not impair spatial learning and memory in the same paradigm, demonstrating that an antidepressant effect can be achieved by SCES, without incurring cognitive side-effects.

An accumulating body of evidence demonstrates that stress decreases the expression of BDNF in brain structures implicated in depression, such as the hippocampus and the PLC (19,29). Chronic antidepressant medications as well as ECT and TMS were found to increase BDNF in these brain areas, adding weight to the hypothesis that BDNF plays an important role in depression and antidepressant treatment (29,55,56). In the present study, BDNF levels were normalized in the dorsal hippocampus and the striatum by repeated SCES or ECT, suggesting a potential therapeutic mechanism, at least for the anhedonic symptom expressed in the CMS model. Interestingly, alterations in BDNF levels induced by CMS and the various treatments were site-specific: BDNF levels were affected in the dorsal but not ventral portion of the hippocampus, and no change was observed in the VTA.

Although several lines of evidence indicate that PFC-striatal interconnection play an important role in the pathophysiology of depression (57), the ventromedial PFC (which is considered functionally parallel to the rat PLC) has not been targeted by DBS for treatment of depression. By contrast, DBS of the ventral striatum (nucleus accumbens) has been recently shown (14) to alleviate anhedonia in treatment-resistant depression. In that

study, however, there was only acute amelioration of depressive symptoms, which reverted to original levels 1 week after withdrawal of stimulation.

Long-term antidepressant effects of brain stimulation were shown usually after repeated sessions (in the case of ECT or TMS) or continued (in the case of DBS) stimulation. The effect of a single stimulation session was not tested in this study, but as with other brain stimulation modalities, it is unlikely that a single brain stimulation session can lead to long-lasting effects.

Although there is no established evidence for functional brain laterality in rat models for depressive behavior, the functional asymmetry between the brain hemispheres and specifically the differential abnormalities in left and right PFC responses in depressive humans are well-documented (23,58). Moreover, TMS has been reported to induce antidepressant effects when high-frequency facilitatory stimulation is applied over the left dorsolateral PFC but, by contrast, when low-frequency inhibitory stimulation is applied over the of right dorsolateral PFC (23). In the present study we tested the lasting effects of left unilateral stimulation, although the effects of bilateral or right unilateral stimulation might warrant future research.

In contrast to DBS and vagal nerve stimulation, where the stimulation is continuous, in this study the temporal parameters of stimulation were more similar to those used in TMS studies. By contrast, the focality of stimulation, with an implanted electrode, resembles more DBS studies in humans.

The long-lasting effects of both DBS and TMS treatments have been explained by alterations in neuroplasticity, including long-term potentiation (LTP)-like and long-term depression-like mechanisms and by gene regulation and receptor modulation mechanisms (59–61). The ability of electrical stimulation to induce short-lasting increases in hippocampal BDNF levels and the role of BDNF in LTP have been previously described (62). Given the accumulating evidence for the role of hippocampal plasticity in depression and in the mode of antidepressant action (29), it is possible that the effects of ECT and SCES are mediated by such LTP and BDNF interaction. The present study demonstrates, firstly, that repeated electrical stimulation of the PLC and NAC induces long-lasting alterations in BDNF levels and, secondly, that this effect is specific to the dorsal hippocampus and the striatum.

Interestingly, although SCES of either the vPLC or the NAC increased sucrose preference and BDNF levels in the dorsal hippocampus and the striatum, the correlation between the measures indicates differential action mechanisms. After vPLC stimulation we found a positive correlation between sucrose preference and BDNF levels in the dorsal hippocampus (and some correlation with BDNF levels in the striatum). By contrast, after NAC stimulation the sucrose preference correlated only with BDNF levels in the striatum but not the dorsal hippocampus. Therefore, it is possible that the antidepressant mechanism of SCES of the NAC involves alteration of striatal BDNF levels, whereas the antidepressant mechanism of SCES of the vPLC involves increases in hippocampal BDNF levels. Additionally, the fact that the same SCES treatments did not affect sucrose preference or BDNF levels in animals that were not exposed to CMS suggests that repeated activation of these pathways (at least when using these parameters) might only normalize impaired neuroplasticity and behavior and reverse the CMS-induced effects but not induce long-lasting facilitation of natural neuroplasticity or behavior.

Overall, the present study suggests that the antidepressant effect of repeated SCES of the vPLC or NAC is comparable to that of ECT, without induction of its associated cognitive deficits. Nevertheless, ECT was found to be more effective in treating anxiety-like behavior induced by CMS. In addition, the present study provides additional support that BDNF in the dorsal hippocampus and the striatum is important in the pathophysiology and treatment of depressive behavior. Further studies would enable a more detailed characterization of stimulation patterns and brain sites in which SCES treatment affects behavioral and neurochemical measures of depression.

Dr. Zangen is an incumbent of the Joseph and Celia Reskin career development chair. This study was supported by the Israel Science Foundation, the Rosenzweig-Coopersmith Fund, and the Feryszka-Sorokin Medical Studies Fund.

Dr. Zangen has financial interest in Brainsway, Inc., a company that develops nonsurgical equipment for transcranial magnetic stimulation. The other authors reported no biomedical financial interests or potential conflicts of interest.

Supplementary material cited in this article is available online.

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