Antidepressant-like Effects of Electroconvulsive Seizures Require Adult Neurogenesis in a Neuroendocrine Model of Depression

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**Abstract**

**Background:** Neurogenesis continues throughout life in the hippocampal dentate gyrus. Chronic treatment with monoaminergic antidepressant drugs stimulates hippocampal neurogenesis, and new neurons are required for some antidepressant-like behaviors. Electroconvulsive seizures (ECS), a laboratory model of electroconvulsive therapy (ECT), robustly stimulate hippocampal neurogenesis.

**Hypothesis:** ECS requires newborn neurons to improve behavioral deficits in a mouse neuroendocrine model of depression.

**Methods:** We utilized immunohistochemistry for doublecortin (DCX), a marker of migrating neuroblasts, to assess the impact of Sham or ECS treatments (1 treatment per day, 7 treatments over 15 days) on hippocampal neurogenesis in animals receiving 6 weeks of either vehicle or chronic corticosterone (CORT) treatment in the drinking water. We conducted tests of anxiety- and depressive-like behavior to investigate the ability of ECS to reverse CORT-induced behavioral deficits. We also determined whether adult neurons are required for the effects of ECS. For these studies we utilized a pharmacogenetic model (hGFAPtk) to conditionally ablate adult born neurons. We then evaluated behavioral indices of depression after Sham or ECS treatments in CORT-treated wild-type animals and CORT-treated animals lacking neurogenesis.

**Results:** ECS is able to rescue CORT-induced behavioral deficits in indices of anxiety- and depressive-like behavior. ECS increases both the number and dendritic complexity of adult-born migrating neuroblasts. The ability of ECS to promote antidepressant-like behavior is blocked in mice lacking adult neurogenesis.

**Conclusion:** ECS ameliorates a number of anxiety- and depressive-like behaviors caused by chronic exposure to CORT. ECS requires intact hippocampal neurogenesis for its efficacy in these behavioral indices.

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**Introduction**

Electroconvulsive therapy (ECT) is the most effective treatment for severe depressive disorders, particularly melancholic depression [1,2]. Successful ECT treatment is associated with normalization of hypothalamic-pituitary-adrenal (HPA) axis abnormalities in this patient population [3,4]. The hippocampus is a limbic brain region that is particularly sensitive to excitotoxic insults that arise from elevated levels of circulating glucocorticoids, and it plays a key role in the stress response by providing negative feedback inhibition over the HPA axis [5–7]. These findings may be clinically relevant because volumetric changes have been noted in the hippocampus of patients with major depressive disorder [8,9]. In the dentate gyrus of the hippocampus, new granule cells are continuously generated throughout adulthood. The process of adult hippocampal neurogenesis includes the proliferation of radial glial-like glial fibrillary acidic protein (GFAP)-positive neural progenitor...
cells and the differentiation of these cells into migrating doublecortin (DCX)-positive neuroblasts. These neuroblasts mature into young granule cells, which integrate into the existing hippocampal circuitry. New neurons have distinct cellular and physiological properties including an increased propensity for excitability and plasticity, which may contribute to their ability to exert influence over mood-related hippocampal circuits [10,11].

Hippocampal neurogenesis is stimulated by chronic treatment with monoaminergic antidepressant drugs. Many research studies have concluded that adult neurogenesis does not contribute to development of depressive-like behaviors per se. However, adult-born neurons may mediate several behavioral effects of pharmacological antidepressant treatments [11–14]. Electroconvulsive seizures (ECS) are a laboratory model of ECT, which stimulate hippocampal neurogenesis [15–18], and counteract the deleterious effects of glucocorticoids on neurogenesis [19]. ECS-induced antidepressant-like behavior and ECS-induced increases in neurogenesis have led to speculation that newborn neurons contribute to the behavioral effects of ECT [20]. However, evidence that newborn neurons are required for ECS to exert antidepressant efficacy has not yet been demonstrated. Here, we investigate this hypothesis in a neuroendocrine mouse model of depression. This model utilizes administration of chronic corticosterone (CORT), and was designed to mimic the HPA axis dysfunction and behavioral disturbances observed in depressed patients [12,21]. The model reliably induces a depression-like state in rodents [12].

Materials and methods

Corticosterone administration

CORT (Sigma–Aldrich) was dissolved in vehicle (0.45% hydroxypropyl-β-cyclodextrin, β-CD) (Sigma–Aldrich), and CORT (35 μg/ml/d) or vehicle was administered to animals ad libitum via the drinking water. Bottles were covered with foil to protect them from light and solutions were freshly made and changed every third day to prevent possible degradation.

ECS treatment

The ECS paradigm consisted of 7 ECS sessions across a 15d period delivered with an Ugo Basile pulse generator (model #57800-001, shock parameters: 100 pulse/s frequency, 3 ms pulse width, 1 s shock duration and 50 mA current). Mice were administered isoflurane anesthesia prior to ECS sessions, and remained anesthetized for the procedure. The stimulation parameters were chosen because they reliably induced tonic-clonic convulsions, caused robust antidepressant behavior and increased hippocampal neurogenesis in our laboratory. No differences in latency to convolution or severity were observed between genotype or treatment groups, and all study animals survived the procedure (data not shown).

Suppression of neurogenesis

To suppress neurogenesis, we utilized mice expressing herpes simplex thymidine kinase (HSV-tk) under control of the human glial fibrillary acidic protein (GFAP) gene promoter (hGFAPtk mice). This pharmagenetic model allows new neurons to be selectively ablated in adulthood [22]. hGFAPtk animas were maintained on a C57Bl6/J background and derived from female hGFAPtk heterozygote X male C57Bl6/J animals (Jackson Labs). In this transgenic model, HSVtk is selectively expressed in GFAP-expressing cells. In the presence of valganciclovir (VGCV), the L-valyl ester of ganciclovir, only those actively dividing GFAP-expressing cells (e.g. neural progenitors; not astrocytes) are ablated [22]. VGCV (Roche) was administered in the animals’ chow (15 mg/kg/d). VGCV-fed wild-type (Ctrl) and hGFAPtk transgenic (NG-) animals were used for these studies. Numerous previous studies have shown that this dose of VGCV reliably leads to absence of all DCX+ cells in the hippocampus. In this study, absence of neurogenesis was confirmed in NG− mice by assessing coronal sections spanning the length of the hippocampus for absence of expression of doublecortin (DCX), a marker of migrating neuroblasts, in both NG−/Sham and NG−/ECS study animals (data not shown).

Behavior tests

To assess for a depressive-/anxiety-like state we examined several previously described measures [14]. Specifically, we assessed behavior in the novelty suppressed feeding test (NSF) and the splash-grooming test. We also conducted an investigation of the animals’ coat state. These specific tests were chosen based on previous studies demonstrating these measures as robustly affected in the neuroendocrine CORT model and as dependent on neurogenesis for an antidepressant response [12–14].

Briefly, the NSF is a conflict anxiety test where motivation to eat competes with fear of a brightly lit arena. Decreased latency to feed is indicative of less anxiety-/depressive-like behavior, while increased latency to feed is indicative of higher anxiety-/depressive-like behavior. Chronic, but not acute administration of monoaminergic antidepressant drugs decreases the latency to feed in the NSF, an effect that requires adult neurogenesis [12–14]. The NSF test was performed during a 15 min period essentially as described [12–14]. The test was conducted by an investigator blind to treatment and genotype groups.

In the splash-grooming test, 200 μL of a 10% sucrose solution was squirted on the mouse’s snout and the latency to groom was recorded as described [12]. Lower latencies to groom are indicative of lower levels of depression/anxiety in this test. The test was conducted by an investigator blind to treatment and genotype groups.

Changes in coat state were assessed by a blinded scorer of five body parts (head, neck, dorsal/ventral coat, tail and paws). For each area, a score of 0 indicated a well-groomed coat and a score of 1 indicated an unkempt coat [12,14]. Scores from the five body parts were summed. Lower summed scores are associated with lower anxiety and depressive-like states, whereas higher summed scores are associated with higher anxiety and depressive-like states.

Tissue processing and immunohistochemistry

Animals were anaesthetized with isoflurane and perfused transcardially with 4% paraformaldehyde. Brains were post-fixed for 16 h and transferred to 30% sucrose. 50 μm sections were cut coronally, mounted in consecutive order onto glass slides and coverslipped using a glycerin-based medium. Sections were systematically sampled 480 μm apart into 12 wells of a 24 well plate. Free-floating sections were washed, blocked and incubated with primary antibody for DCX (sc–8066, 1:250, SCBT) overnight, washed and stained with biotinylated donkey anti-goat secondary antibody (1:500, Life Technologies) with 10% normal donkey serum. Endogenous peroxidase activity was blocked using 0.3% hydrogen peroxide for 30 min at room temperature. The horseradish peroxidase (HRP)-3,3′-diaminobenzidine (DAB) reaction was carried out using an avidin/biotin peroxidase complex (VectaStainABC Kit, Vector Laboratories). Sections were incubated in the provided ABC solution for 1 h and DAB (Sigma–Aldrich) for 3 min. Every 12th section through the hippocampal dentate gyrus was identified and all doublecortin positive cells bodies were counted. Analysis was
performed as previously described to count the total number of DCX+ cells and to measure the extent of dendritic branching of DCX+ cells [12].

**Study design**

Figure 1A displays a timeline for experiments conducted in Figs. 1 and 2. In these experiments, CORT or vehicle (β-CD) was administered to 8 week-old, male, wild-type animals in the drinking water for 4 weeks before initiating ECS or Sham treatment. CORT treatment continued throughout the ECS treatment, and was removed from the drinking water after the final ECS session. Behavior experiments or animal perfusion for histology experiments were conducted 48 h following the final ECS session. Behavior experiments were conducted on four groups: Vehicle/Sham (n = 12), Vehicle/ECS (n = 12), CORT/Sham (n = 11), CORT/ECS (n = 12). One animal was excluded from the Vehicle/Sham group in the NSF test due to an experimental problem. One animal in the Vehicle/ECS and one animal in the CORT/ECS group were excluded due to mild dermatitis making reliable coat scoring difficult. Histology experiments were conducted on a separate cohort of four groups: Vehicle/Sham (n = 6), Vehicle/ECS (n = 6), CORT/Sham (n = 5), CORT/ECS (n = 6).

Figure 3A displays a timeline for experiments conducted in Fig. 3. In brief, 8 week-old, male wild-type (Ctrl) and animals without neurogenesis (NG-) began VGCV treatment 4 weeks prior to initiation of CORT administration. VGCV treatment continued for the remainder of the experiment. After 4 weeks of CORT administration, ECS treatment was initiated. CORT treatment continued throughout the ECS treatment, and was removed from the drinking water after the final ECS session. Behavior experiments or animal perfusion for histology experiments were conducted 48 h following the final ECS session. Behavior and histology experiments were conducted on four CORT-treated groups: Ctrl/Sham (n = 9), Ctrl/ECS (n = 7), NG-/Sham (n = 8), NG-/ECS (n = 9). One animal in the NG-/ECS group was excluded due to an experimental problem. All procedures were
conducted according to NIH guidelines and approved by the Institutional Animal Care and Use Committee of the National Institute of Mental Health and/or the Johns Hopkins University.

Statistical analysis

Experimental results in the graphical presentations and tabular results are presented as the mean ± SEM. Comparisons of behavior and weight data were analyzed using a 2-way ANOVA with Newman–Keuls post-hoc tests in Prism 6 software (GraphPad). Comparisons of immunohistochemical data were performed using a 2-way ANOVA with Bonferroni-Dunn post-hoc tests with Statview software. Summary data is presented in graphical format (Figs. 1–3) and raw data and statistical information is presented in Tabular format (Table 1).

Results

ECS rescues behavior in a neuroendocrine model of depression

In this study we compared the behavioral effects of Sham versus ECS in the neuroendocrine model of CORT-induced depressive-/anxiety-like behavior. As expected, CORT treatment had a significant effect on latency to feed in the NSF test, suggesting higher depressive-/anxiety-like behavior in the neuroendocrine model of depression. ECS was able to reverse the depressive/anxiety-like state observed in the NSF test after CORT treatment (Fig. 1B, Table 1). We next assessed the coat state of the animals, a well-validated index of a depressed-like state. As expected, ECS significantly improved the condition of the fur. This beneficial effect of ECS was blocked in grooming behavior by squirting a 10% sucrose solution on the mouse’s snout and assessing the latency to groom. CORT treatment had a significant effect on the latency to groom in this test, an effect that was reversed by ECS treatment (Fig. 1D, Table 1). In line with previous studies, CORT treatment slightly increased overall body weight, and ECS led to significant weight decreases in both the vehicle and CORT-treated animals (Fig. 1E, Table 1).

ECS robustly increases measures of neurogenesis

In this study we investigated the effects of ECS in vehicle and CORT-treated animals on measures of hippocampal neurogenesis. ECS significantly increased the total number of migrating neuroblasts (DCX+ cells) in both vehicle and CORT-treated animals (Fig. 2A and B, Table 1). We also assessed the effects of ECS on the dendritic maturation of newly born cells by examining the dendritic morphology of DCX+ cells. ECS significantly increased the number of DCX+ cells containing tertiary dendrites in both vehicle and CORT-treated animals (Fig. 2A and C, Table 1).

New neurons are required for anti-depressive activity of ECS in the neuroendocrine model of depression

In this study, we compared the behavioral effects of ECS in CORT-treated wild-type (Ctrl) animals versus CORT-treated animals lacking neurogenesis (NG-). As expected, ECS significantly decreased the latency to approach in the NSF test in Ctrl animals, suggesting antidepressant activity of ECS. However, the antidepressant effect of ECS was blocked in NG-animals (Fig. 3B, Table 1). Next, we assessed the effect of ECS on coat state in CORT-treated Ctrl animals, and found that ECS significantly improved the condition of the fur. This beneficial effect of ECS was blocked in...
NG-animals (Fig. 3C, Table 1). Next, we examined the latency to groom in the splash test, and found that ECS significantly decreased the latency to groom in Ctrl animals, indicative of an antidepressant effect of ECS. Again, this effect of ECS was blocked in NG-animals (Fig. 3D, Table 1). As in our previous experiment, a significant decrease in weight was observed after ECS; however, this effect was not affected by loss of neurogenesis (Fig. 3E, Table 1).

Discussion

The mechanisms by which ECT ameliorates depression in humans are not completely understood. ECS enhances adult hippocampal neurogenesis in both rodent and non-human primate models [15–18]. To our knowledge, this report is the first to describe evidence that adult born neurons contribute to the behavioral effects of ECS. Specifically, our data show that the antidepressant-like activity of ECS in a neuroendocrine rodent model of depression requires adult hippocampal neurogenesis. Interestingly, changes in neurogenesis can impact inhibitory control of the hippocampus over the HPA axis [23–25]. While our study design did not allow for meaningful HPA axis measurements due to delivery of exogenous glucocorticoids, future studies should examine whether improvements in neurogenesis induced by ECS can normalize stress-induced HPA axis abnormalities. Such experiments are of interest since they link two hypotheses regarding the mechanisms underlying ECT efficacy, e.g., HPA axis normalization and enhanced neuroplasticity due to increased neurogenesis [20].

Reliable protocols for in vivo measurement of neurogenesis are not yet available in humans, but several promising technologies are under development [11]. Our results suggest that measurements of adult neurogenesis could serve as a potential biomarker to predict and monitor response to ECT in the future. Demonstrating a causal relationship between increased neurogenesis and ECS efficacy is also important pre-clinically. Our findings establish a foundation for future studies aimed at deciphering the cellular mechanisms by which adult born neurons impact hippocampal circuitry to ameliorate depressive-like behavior after ECS treatment. Such experiments are of high importance because understanding the biological mechanisms by which ECT exerts its antidepressant action are crucial to develop improved treatments. Specifically, these
| Sapsolky RM. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Arch Gen Psychiatry 2000;57(10):925–35.
| Bolwig TG, Madsen TM. Electroconvulsive therapy in melancholia: the role of neuroplasticity in multiple brain regions. Our data suggest that enhancement of hippocampal neurogenesis may functionally contribute to the antidepressant effects conferred by ECT. Because ECT remains the most effective clinical treatment for both severe and treatment resistant depression, understanding the underlying mechanisms is of paramount importance.

**References**

5. [Sapsolky RM. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Arch Gen Psychiatry 2000;57(10):925–35.](#)

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