Brain Stimulation 8 (2015) 862–867

Contents lists available at ScienceDirect

Brain Stimulation

journal homepage: www.brainstimjrnl.com

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

Robert J. Schloesser [a,b], Sophie Orvoen [c], Dennisse V. Jimenez [b], Nicholas F. Hardy [b], Kristen R. Maynard [b], Mahima Sukumar [b], Husseini K. Manji [d], Alain M. Gardier [c], Denis J. David [c], Keri Martinowich [b,e,*]

[a] University of Maryland School of Medicine, Department of Psychiatry, Baltimore, MD, USA
[b] Lieber Institute for Brain Development, Johns Hopkins Medical Campus, Baltimore, MD, USA
[c] Université Paris Sud, INSERM UMR S 1178, Faculté de Pharmacie, Châtenay-Malabry, France
[d] Global Therapeutic Area Head, Neuroscience, Janssen Research & Development, Titusville, NJ, USA
[e] Departments of Psychiatry and Neuroscience, Johns Hopkins School of Medicine, Baltimore, MD, USA

ARTICLE INFO

Article history:
Received 11 January 2015
Received in revised form
5 May 2015
Accepted 31 May 2015
Available online 29 June 2015

Keywords:
ECT
ECS
Hippocampus
Neurogenesis
Neuroplasticity
Antidepressant

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.

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 glia-like glial fibrillary acidic protein (GFAP)-positive neural progenitor

Funding for this study was provided in part by the Intramural Program of the National Institute of Mental Health, the former affiliation of RJS, DVJ, NFH, HKM and KM. Additional funding was provided by the Lieber Institute for Brain Development and the Brain Behavior Research Foundation (NARSAD Young Investigator Awards to RJS, KM).

* Corresponding author. Lieber Institute for Brain Development, 855 N. Wolfe Street, Suite 300, Baltimore, MD 21205, USA. Tel.: +1 410 955 1510.
E-mail address: keri.martinowich@libd.org (K. Martinowich).

http://dx.doi.org/10.1016/j.brs.2015.05.011
1935-861X/

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 inhaled 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 convulsion 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 pharmacogenetic 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