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Gadd45b Mediates Electroconvulsive Shock Induced Proliferation of Hippocampal Neural Stem Cells

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Abstract

Background—Electroconvulsive shock (ECS), also known as an electroconvulsive therapy (ECT), is an effective and safe treatment for neuropsychiatric disorders including pharmacoresistant major depressive disorder. Previous research in animal models suggests ECS efficacy is achieved by Gadd45b-mediated increases in adult hippocampal neurogenesis.

Objective/Hypothesis—The present study aims to delineate the role of Gadd45b in mediating proliferation of neural stem cell types including quiescent radial glia-like (RGL) and amplifying non-radial glia-like (non-RGL) neural precursors following ECS.

Methods—RGL and non-RGL neural stem cell populations defined by co-localization of $MCM2^+$ and $nestin^+$ cells and morphologically by the presence of radial processes were stereologically analyzed.

Results—ECS increased hippocampal density of both quiescent RGLs and amplifying non-RGLs.

Conclusions—Gadd45b mediates the action of ECS-induced proliferation through activation of quiescent neural stem cells.

Keywords

Electroconvulsive shock; Gadd45b; Adult neurogenesis; Neural stem cells; Hippocampus

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Introduction

Electroconvulsive therapy (ECT) is effective in treating pharmacoresistant major depressive disorder and other neuropsychiatric disorders [1,2]. While the mechanisms mediating ECT efficacy remain largely unknown, significant evidence supports adult hippocampal neurogenesis as a neurobiological mechanism [3–5]. Using rodent and non-human primate models treated with analogous electroconvulsive shock (ECS), studies show ECS treatment increases adult hippocampal neurogenesis [5–7].

Adult hippocampal neurogenesis is the process wherein adult-born neurons arising from neural stem cells (NSCs) contribute to memory, cognition, and mood regulation [6–8]. The subgranular zone (SGZ) of the adult dentate gyrus includes the following two major hippocampal NSC types: 1) Radial glia-like cells (RGLs) exhibiting mitotic quiescence and radial processes extending towards the molecular layer. 2) Amplifying non-radial glia-like precursors (non-RGLs) lacking radial processes and which undergo increased mitotic proliferation relative to RGLs. Activation of quiescent RGLs results in asymmetric division and subsequent production of self-renewing amplifying non-RGLs [8,9]. Interestingly, previous work demonstrates ECS promotes RGL proliferation and accelerates their maturation towards newborn dentate granule neurons, correlating with behavioral benefits [7,10–12]. Significant increases in neurogenesis in the dentate gyrus or downstream effector molecules such as β-catenin may be observed as early as 3 days or 1 day respectively, following acute ECS treatment [3,13]. While the molecular mediators linking ECS action on these NSC developmental events are not fully understood, our previous work implicates growth arrest and DNA-damage-inducible protein 45 beta (Gadd45b) as a potential mechanism mediating ECS action [13].

Gadd45b is a stress response gene causing long-lasting changes in hippocampal neurogenesis when its expression is transiently induced in response to neuronal activity [13]. Acting as an immediate early gene in dentate granule cells, its expression rapidly increases following ECS or physiological stimulation such as exercise. Both exercise and ECS increase proliferation of neural progenitors and post-mitotic dendritic growth of newborn neurons, effects that are significantly attenuated in mice lacking Gadd45b [13]. Furthermore, Gadd45b mediates active DNA demethylation of specific regulatory promoter regions and expression of corresponding genes critical in regulating adult neurogenesis such as genes for brain derived neurotrophic factor (*bdnf*) and fibroblast growth factor 1 (*fgf1*) following ECS and exercise, providing evidence supporting a neurotrophic mechanism for its proliferative effects [13].

While implicated in NSCs, the function of Gadd45b in mediating RGL proliferation remains unknown. Here we demonstrate quiescent adult hippocampal NSCs defined by co-expression of minichromosome maintenance complex component 2 (MCM2), an endogenous marker for cell proliferation, and nestin, a NSC marker, respond to ECS contingent on the presence of Gadd45b.

Methods and materials

Animals and ECS treatment

8–10 week old female wild-type (WT) and Gadd45b knockout (KO) mice littermates with C57BL/6 background were used (Charles River). Sample sizes and grouping were based on previous study showing significant differences following ECS [13]. Animals received a single ECS via ear-clip electrodes using the Ugo Basile ECS unit (Model 7801) as previously described [13,14]. Under the specific parameters (1.0 s, 100 Hz, 18 mA stimulus of 0.3 ms square wave pulses), animals initially exhibited full hind limb extension for 1–2s with an overall seizure duration of > 20s and full recovery in < 5 min [13,14]. Both Gadd45b WT and KO mice showed similar convulsions. Sham animals were similarly handled in parallel without ECS. Three days later, mice were sacrificed, fixed, and brains subjected to analysis. Previous studies show a single ECS treatment induces significant proliferative effects visible at this time point [5,13]. All experimental procedures were performed in accordance with the animal protocol approved by the Institutional Animal Care and Use Committee.

Histological procedures and stereological analysis

Coronal brain sections (40 μm) were immunostained using an antigen retrieval procedure as previously described [14]. Immunostaining was performed with the following primary antibodies: anti-nestin (Aves; chick; 1:500 dilution), and anti-MCM2 (BD; mouse; 1:500 dilution). 4',6-diamidino-2-phenylindole (DAPI; Sigma) was used for counterstaining. Images were acquired on a LSM 780 confocal system (Carl Zeiss) with X40 objective lens and multi-track configuration. Stereological quantification of SGZ $MCM2^+$ and $MCM2^+nestin^+$ cells was carried out as previously described [14]. RGLs were considered $MCM2^+nestin^+$ cells with apical process. Non-RGLs were considered $MCM2^+nestin^+$ cells with small horizontal processes or no processes [9,15]. An observer blind to the genotypes and treatment of the animals performed assessments. Statistical significance ($P < 0.05$) was assessed by one-way ANOVA.

Results

To test the role of Gadd45b in ECS-induced neural progenitor proliferation, we counted $MCM2^+$ cells within the SGZ. Stereological quantification showed similar $MCM2^+$ cell densities between WT and KO mice without ECS (Figs. 1A and 1B), indicating Gadd45b does not affect basal neural progenitor proliferation. However, with ECS, there was a significant increase in $MCM2^+$ cell density in WT mice. This effect was completely abolished in Gadd45 KO mice, suggesting Gadd45b is required for ECS-induced proliferation of neural progenitors.

To further determine the role of Gadd4b, we assessed NSC subtypes, RGL and non-RGL, in response to ECS. NSC subtypes were identified by co-localization of $MCM2^+$ and $nestin^+$ cells with radial processes extending towards the molecular layer (RGLs), and with non-radial processes within the adult SGZ (non-RGLs) (Fig. 1C and Supplementary Movies 1 and 2). Stereological quantification revealed ECS of WT mice markedly increased both

RGL and non-RGL populations, but such effect was significantly diminished in their KO littermates (Fig. 1D). Taken together, these results demonstrate a specific and crucial role of Gadd45b in ECS-induced, but not basal, proliferation of RGLs and non-RGLs in the adult dentate gyrus.

Discussion

In the present study, we identify the novel function of Gadd45b as a key mediator regulating ECS-induced proliferation of RGL and early stage non-RGL NSCs. Previous research shows ECS-induced proliferation primarily stems from activated quiescent RGLs [7,10]; however, the role of Gadd45b in this activation remained unexplored. We found ECS induced proliferation of both RGLs and non-RGLs, an effect that was abolished in Gadd45b KO mice, demonstrating the requirement of Gadd45b in activating RGLs in response to ECS. While the mechanism is not fully known, previous studies associate BDNF and FGF increases with neurogenesis *in vitro* and *in vivo* [13,16–19]. Similarly, our previous study suggests that Gadd45b is essential niche component in ECT-induced DNA-demethylation in the specific promoter regions of BDNF and FGF1 mediating adult hippocampal neurogenesis [13]. Thus, Gadd45b activity-dependent demethylation of these same genes may underlie RGL activation following ECS.

While the present study analyzed a molecular mechanism that suggests changes in hippocampal neurogenesis driving ECT efficacy, other mechanisms may mediate general antidepressant action [20]. Where antidepressants such as the selective serotonin-reuptake inhibitor fluoxetine require weeks for depression relief, lending support to the neurogenic hypotheses and the role of serotonin in neurogenesis [24], fast-acting antidepressants such as the NMDA antagonist ketamine act within hours and are effective where conventional therapies are not [21]. One proposed mechanism is mTOR mediated rapid alterations in synaptic connectivity between the hippocampus and medial prefrontal cortex [22,23]. In light of the many hypotheses proposed for antidepressant efficacy, the true mechanism is likely multifactorial in nature. Future research will need to isolate the major therapeutic contributors for the development of highly specific and effective antidepressants.

This is the first study showing Gadd45b controls proliferation of RGL and non-RGL NSCs in response to ECT-induced neural activity. Given a substantial role for adult hippocampal neurogenesis in mediating ECS efficacy and associated functional improvements [12], targeting Gadd45b regulation of the NSC pool may represent a new therapeutic strategy for major depression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- ECS promotes proliferation of quiescent radial-like (RGLs) neural stem cells and amplifying non-RGL precursors in the adult mouse dentate gyrus.
- Gadd45b does not affect basal proliferation level of RGLs and non-RGL precursors in the adult mouse dentate gyrus.
- Gadd45b is essential for ECS-induced proliferation of RGLs neural stem cells and non-RGL precursors in the adult mouse dentate gyrus.

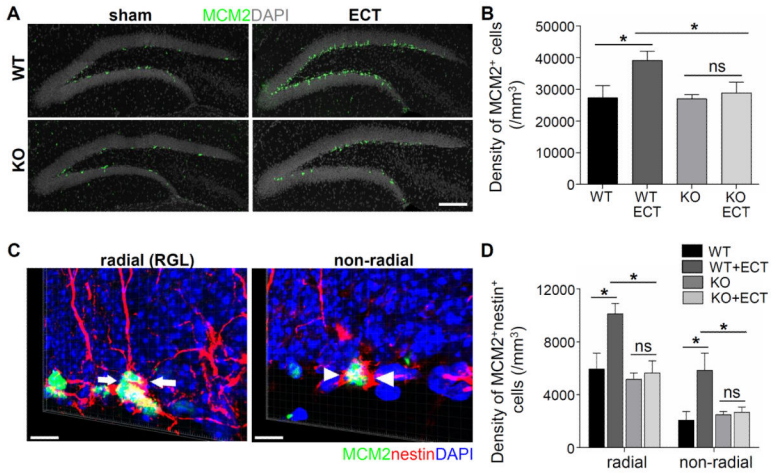

Figure 1. Crucial role of Gadd45b in electroconvulsive shock (ECS)-induced proliferation of quiescent radial glia-like (RGLs) neural stem cells and non-RGL neural precursors

(A–B) Gadd45b mediates ECS-induced proliferation of neural stem cells in the subgranular zone (SGZ). **A**. Representative confocal images of immunostaining of MCM2 (green), an endogenous cell proliferation marker, and DAPI staining (grey) in the dentate gyrus of adult Gadd45b knockout (KO) and wild-type (WT) littermates with or without ECS. Scale bar: 50 μm. **B**. Summary of stereological quantification of $MCM2^+$ cells in the adult dentate gyrus of WT and KO littermates with or without ECS. Values represent mean ± SEM (n = 4 animals per group; *P < 0.05, one-way ANOVA; ns, no significance). **(C–D)** Gadd45b mediates ECS-induced activation of RGL and non-RGL neural stem cells in the SGZ. **C**. Sample confocal images of immunostaining of MCM2 (green), nestin (red) and DAPI staining (blue). Scale bars: 25 μm. Arrows point to $MCM2^+nestin^+$ RGL (left; arrows) and $MCM2^+nestin^+$ non-RGL neural precursors (right; arrowheads), respectively (See Supplementary Movie 1). **D**. Summary of stereological quantification of $MCM2^+nestin^+$ RGL and non-RGL precursors in the adult dentate gyrus of WT and KO littermates with or without ECS. Values represent mean ± SEM (n = 4 animals per group; *: P < 0.05, one-way ANOVA; ns, no significance).