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Research Report

Effects of single and repeated electroconvulsive stimulation on hippocampal cell proliferation and spontaneous behaviors in the rat

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ABSTRACT

Electroconvulsive therapy (ECT) has therapeutic effects on refractory depression and schizophrenia, although its biological mechanisms are still unclear. Recent studies in rodents suggest that electroconvulsive stimulation-induced seizures (ECSs) influence hippocampal adult neurogenesis, which has gained considerable traction as a possible cellular substrate for the treatment of depression. The aim of this study is to explore alteration of neurogenesis in the hippocampus following ECSs and the relationship between neurogenesis and behavior in rats. In the present study, we administered a single or 10-repeated application of electroconvulsive stimulations that reliably resulted in seizure (an animal model of electroconvulsive therapy) to rats. Then cell proliferation of newborn cells in the subgranular zone (SGZ) of the dentate gyrus (DG) was investigated 3 and 14 days after ECS treatments. Cell differentiation was also examined 4 weeks after newly formed cells were confirmed. As a result, ECS-induced cell proliferation in the hippocampus showed biphasic changes after ECS. The amount of cell proliferation at 3 days after the last ECS increased twice as much as the sham group. However, the number of proliferating cells at 14 days later decreased to a half of the sham level. Differentiation of newly formed cells was not influenced in ECS-treated groups compared with sham-treated groups. In addition, we investigated the effects of ECS on behavioral changes in rats by measuring locomotor activity in an open field test and spontaneous alteration behavior in a Y-maze test. Spontaneous behavior and memory function were not influenced by repeated ECSs. These results suggest that repeated ECSs affect progenitors that have a limited ability for cell proliferation, like amplifying progenitors, to increase newly generated neurons without negative behavioral change.

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1. Introduction

Human imaging and postmortem studies of depression have implicated several brain regions such as the prefrontal and cingulate cortex, hippocampus, striatum, amygdala and thalamus, in which dysfunction may cause diverse symptoms of depression (Drevets, 2001; Liotti and Mayberg, 2001; Nestler et al., 2002). Several classic studies provided a foundation for the idea that the hippocampus is involved in the regulation of mood by demonstrating the vulnerability of the hippocampus to various hormones induced by stressful experience (McEwen, 1999). For example, 21 days of restraint stress or corticosterone treatment leads to atrophy of apical dendrites in the CA3 subfield of the hippocampus (McEwen et al., 1995; McEwen, 1999; Samuels and Hen, 2011).

Adult neurogenesis, the birth of neurons in the adult animal, has been observed throughout life in mammals, including humans, in two discrete regions, the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the dentate gyrus (DG) in the hippocampus (Ming and Song, 2005). The proliferation, differentiation, and survival of dentate granule neurons are influenced by various physiological and environmental conditions (Kempermann et al., 1997; van Praag et al., 1999). Exposure to different forms of chronic stress, including social defeat, physical restraint and footshock can inhibit adult neurogenesis in multiple species (Czeh et al., 2002; Gould et al., 1998). Glucocorticoids also inhibit the proliferation and differentiation of neural progenitors and the survival of young neurons (Wong and Herbert, 2004).

Specifically, neurogenesis in the DG has gained considerable traction as a cellular substrate underlying the treatment of depression. In most studies, however, ablation of hippocampal neurogenesis alone is not sufficient to induce a phenotype reminiscent of either anxiety or depression (Airan et al., 2007; Holick et al., 2008; Sahay and Hen, 2007; Santarelli et al., 2003; Zhao et al., 2008). There is a requirement for adult neurogenesis in mediating some of the beneficial effects of antidepressants, while decreasing neurogenesis alone is not sufficient to drive a depression-like phenotype (Samuels and Hen, 2011; Santarelli et al., 2003). Mice that have been exposed to focal X-irradiation of the hippocampus, resulting in a loss of adult hippocampal neurogenesis, do not show this response to either fluoxetine or imipramine (Santarelli et al., 2003). Chronic antidepressant treatment increases adult neurogenesis in the DG as measured by uptake of bromodeoxyuridine (BrdU) in the SGZ (Malberg et al., 2000). In addition to increasing proliferation in the DG, chronic fluoxetine accelerates the maturation of young neurons by increasing arborization (Wang et al., 2008). However, much less is known about why the increase in neurogenesis is required for the antidepressant response. It is possible that young neurons may contribute to hippocampal-dependent negative feedback of the HPA axis through stimulation of the subiculum, CA3 or DG (Dunn and Orr, 1984).

Electroconvulsive therapy (ECT) is known for its successful effects on severe depression. Although the history of this therapy is long, the biological mechanism underlying its beneficial effects remains largely unknown. In animal models of ECT, acute electroconvulsive stimulation-induced seizure (ECS) is also effective at increasing neurogenesis (Ito et al., 2010; Madsen et al., 2000; Malberg et al., 2000; Manev et al., 2001). We have previously examined whether a certain number of ECSs yielded maximum stimulation of hippocampal cell proliferation. The result was that repeated ECS for 10 days (once a day) had the greatest effect on increasing neurogenesis (Ito et al., 2010). In clinical settings, 6–12 electroconvulsive therapies achieve the desired result in the majority of melancholics (Abrams, 2002). However, some studies have demonstrated that repeated ECSs caused negative behavioral changes. Hidaka et al. (2008) showed repeated ECS could cause impairment of spontaneous alternative behavior in the Y-maze, which assesses short term memory, and increase in the locomotor activity of rats in the open field test (Hidaka et al., 2008).

In this study, we administered a single or 10-repeated application of ECS to rats. Then proliferation of newborn cells in SGZ of the DG was investigated at 3 and 14 days after ECS treatments. Cell differentiation was also examined 4 weeks after newly formed cells were confirmed. In addition, we investigated the effects of ECS on behavioral changes in rats by measuring locomotor activity in an open field test and spontaneous alteration behavior in a Y-maze test and examined whether those effects were neurogenesis-dependant.

2. Results

2.1. *Effects of ECS administration on cell proliferation*

All rats treated by ECS were administered BrdU to label proliferating cells 24 h prior to a decapitation. Before cell counting, BrdU-positive cells were typically observed in the DG. No differences in gross morphology or location of BrdU-positive cells were observed between ECS treated and sham groups.

Effects of ECS treatment of cell proliferation were examined using two-way ANOVA. At 3 days after the last ECS administration, statistical analysis showed significant main effects of both “treatment with ECS or sham” (F [1, 23]=116.3, $p=1.81\times10^{-10}$) and “number of ECSs” (F [1, 23]=19.7, $p=1.90\times10^{-4}$) on the number of BrdU-positive cells in the SGZ (Fig. 2A). An interaction between these two effects was also significant (F [1, 23]=8.93, $p=6.57\times10^{-3}$). Post-hoc analysis using Bonferroni’s test revealed significant increases in cell proliferation in the SGZ in both single and 10 repeated ECSs groups by +84% and +130% compared with each sham-treated group, respectively ($p=1.62\times10^{-5}$, $p=8.19\times10^{-10}$). In addition, rats that received 10 repeated ECSs displayed a significant increase by +43% in BrdU-positive cells compared with rats that received a single ECS ($p=2.40\times10^{-5}$).

In 14 days after the last ECS, there were significant main effects of “treatment with ECS or sham” (F [1, 20]=87.5, $p=9.61\times10^{-9}$) and “number of ECS administration” (F [1, 20]=9.52, $p=0.00583$) (Fig. 2B). An interaction between these two effects was not significant (F [1, 20]=0.026, $p=0.873$). Multiple comparison test revealed that cell proliferations in both the single and 10 times ECSs were decreased by −52% and −62%