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IMPACT OF HIPPOCAMPAL NEURONAL ABLATION ON NEUROGENESIS AND COGNITION IN THE AGED BRAIN

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Abstract—Neuronal loss is the most common and critical feature of a spectrum of brain traumas and neurodegenerative disorders such as Alzheimer's disease (AD). The capacity to generate new neurons in the central nervous system diminishes early during brain development and is restricted mainly to two brain areas in the mature brain: subventricular zone and subgranular zone. Extensive research on the impact of brain injury on endogenous neurogenesis and cognition has been conducted primarily using young animals, when neurogenesis is most active. However, a critical question remains to elucidate the effect of brain injury on endogenous neurogenesis and cognition in older animals, which is far more relevant for age-related neurodegenerative disorders such as AD. Therefore, we examined the impact of neuronal loss on endogenous neurogenesis in aged animals using CaM/Tet-DT$_A$ mice, a transgenic model of hippocampal cell loss. Additionally, we investigated whether the upregulation of adult neurogenesis could mitigate cognitive deficits following substantial hippocampal neuronal loss. Our findings demonstrate that aged CaM/Tet-DT$_A$ mice that sustain severe neuronal loss exhibit an upregulation of endogenous neurogenesis. However, despite this significant upregulation, neurogenesis alone is not able to mitigate the cognitive deficits observed. Our studies suggest that the aged brain has the capacity to stimulate neurogenesis post-injury; however, multiple therapeutic approaches, including upregulation of endogenous neurogenesis, will be necessary to recover brain function after severe neurodegeneration. © **2014 Published by Elsevier Ltd. on behalf of IBRO.**

Key words: neurogenesis, neuronal loss, hippocampus, neurodegenerative disorders.

INTRODUCTION

Neuronal loss is a common characteristic of a wide spectrum of brain traumas such as stroke, epilepsy, and traumatic brain injury (TBI), as well as neurodegenerative disorders such as Alzheimer's, Parkinson's, and Huntington's disease (Lunn et al., 2011). The incidence and prevalence of these traumas and brain disorders are rising due to the increasing life expectancy of the human population (Salomon et al., 2012). Additionally, Alzheimer's disease (AD) prevalence is expected to increase extensively in the USA, rising from 5.4 million to 13.8 million by 2050 (Hebert et al., 2001, 2013; Thies and Bleiler, 2013). Given the overwhelming number of people that will suffer from brain traumas and neurodegenerative disorders, we urgently need a better understanding of the endogenous mechanisms that follow neuronal loss in order to target these mechanisms for therapeutic benefit.

Neurogenesis is a critical mechanism during brain development that may also contribute to spatial and contextual memory formation in the adult brain (van Praag et al., 2002; Deng et al., 2010; Sahay et al., 2011; Pan et al., 2013). Neurogenesis in the mammalian brain is diminished early during brain development with the exception of two neurogenic regions: subventricular zone (SVZ) and subgranular zone (SGZ) (Eriksson et al., 1998; Gage, 2000; Taupin and Gage, 2002). Neurogenesis in the SVZ is generated from neural stem/progenitor cells (NSCs) that differentiate into granule and periglomerular cells (Altman, 1969). Conversely, neurogenesis in the SGZ is generated from NSCs that differentiate into neuronal and glial cells in the granular layer (Altman and Das, 1965; Cameron et al., 1993). Previous studies have shown an imperative role for neurogenesis in the SGZ for spatial and contextual memories, demonstrating an important link between adult neurogenesis and cognition (van Praag et al., 2002; Sahay et al., 2011). In addition, a recent study has shown that various forms of learning promote the survival of newborn neurons in the SGZ. (Curlik et al., 2013). Overall, these studies reveal an important role of neurogenesis in the cognitive processes.

Here we sought to assess changes in neurogenesis specifically following neuronal loss in aged animals, in order to recapitulate the cell loss found in the adult human population with neurodegenerative disorders and

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Abbreviations: AD, Alzheimer's disease; BrdU, 5-bromo-2-deoxyuridine; CamKIIα, calcium-calmodulin kinase II alpha; CE, coefficient of error; DCX, doublecortin; DG, dentate gyrus; DT$_A$, diphtheria toxin A chain; EdU, 5-ethynyl-2-deoxyuridine; PBS, phosphate-buffered saline; SGZ, subgranular zone; SVZ, subventricular zone; TBS-T, Tween-20–Tris-buffered saline; TRE, tetracycline-responsive element; tTA, tetracycline-controlled transcriptional activator; Tx-100, Triton X-100; TBI, traumatic brain injury; NSCs, neural stem/progenitor cells.

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brain injuries. We utilized a transgenic mouse model (CaM/Tet-DT_A mouse model) that allows for selectively induced neuronal loss without invasive techniques that may damage other brain areas. Thus, this transgenic model provides us with a useful tool to study the role of neurogenesis in the adult brain after selective neuronal loss. The CaM/Tet-DT_A mouse model utilizes a Tet-Off inducible transgene system by crossing the TRE-DT_A mice with CaMKIIα-tTA mice, producing a consistent and non-invasive lesion in the CA1 upon removal of doxycycline from the diet (Mayford et al., 1996; Lee et al., 1998). Our results indicate that 21 days of Tet-DT_A induction in aged CaM/Tet-DT_A mice (12 months) causes significant neuronal loss in the hippocampus (including CA1, CA3 and dentate gyrus (DG)). This massive neuronal loss in CaM/Tet-DT_A lesioned mice results in substantial cognitive deficits compared to non-lesioned CaM/Tet-DT_A mice. Interestingly, CaM/Tet-DT_A lesioned mice display a significant upregulation in neurogenesis, both in cell proliferation and cell survival. Overall, these results indicate the aged brain is capable of producing new neurons following severe hippocampal injury; however, an upregulation of neurogenesis alone is insufficient to protect from the cognitive loss associated with severe hippocampal injury.

EXPERIMENTAL PROCEDURES

Transgenic mouse model of neuronal loss

All animal experimental procedures were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) at the University of California, Irvine. All mice were housed with food and water *ad libitum* under a 12-h dark/light cycle. All the mice utilized in this study were 12-month-old CaM/Tet-DT_A mice.

The CaM-Tet-DT_A mouse model, characterized in the lab, utilizes a double transgene system that is capable of inducing cell ablation in the forebrain and specifically in the CA1 region of the hippocampus (Yamasaki et al., 2007). The calcium-calmodulin kinase II alpha (CamKIIα) promoter drives the expression of the first transgene, tetracycline-controlled transcriptional activator (tTA), which binds to the tetracycline-responsive element (TRE) and drives the expression of the second transgene, diphtheria toxin A chain (DT_A). This model allows for an inducible Tet-off system controlled by a doxycycline diet. With doxycycline present, tTA is sequestered, thereby suppressing the DT_A transgene. Upon removal of doxycycline, tTA is can freely bind to the TRE element to allow DT_A expression.

These mice were aged for 12 months from birth, after which doxycycline was removed from their diet for 21 days allowing for a 21-day lesion of the hippocampus and cortex. Post-lesion, mice were given a 1-month period to recover followed by 5 days of 5-bromo-2-deoxyuridine (BrdU) or phosphate-buffered saline (PBS) injections to allow for the visualization of newly developing neurons in the hippocampus. Behavioral assessments were then conducted, followed by 5 days of 5-ethynyl-2-deoxyuridine (EdU) injections, and immediate euthanasia was performed under sodium pentobarbital anesthesia (Fig. 1A).

Bromodeoxyuridine labeling

To label maturing endogenous neuronal stem cells, mice were given a twice-daily intraperitoneal (IP) injection of bromodeoxyuridine at 50 mg/kg (BrdU, Sigma–Aldrich, St. Louis, MO, USA), beginning 1 month post-lesion for 5 consecutive days (Fig. 1A).

Ethynyldeoxyuridine labeling

To label proliferating neuronal cells, the same cohort of mice were given a single daily IP injection of Ethynyldeoxyuridine at 50 mg/kg (EdU, Invitrogen, Grand Island, NY, USA), beginning 5 days prior to euthanasia (Fig. 1A).

Barnes maze

To evaluate spatial learning and memory after inducing neuronal loss, a 5-day Barnes maze protocol was utilized. Briefly, the Barnes maze consists of a 120-cm diameter white disk elevated 120 cm above the floor with 40 holes, 5 cm in diameter, evenly spaced around the parameter. Located beneath one of the holes, serving as the goal box, is a metallic box, 10.5-L $\times$ 6.0-W $\times$ 6.0-H cm, with torn gauze bedding on the base. The Barnes maze was stationary in a room illuminated by standard ceiling lighting with visual cues along the walls for directionality purposes. A video camera recorder was stationed approximately 30 cm from the apparatus and recorded from an angle to allow for performance monitoring.

The mice were trained for 4 days and underwent a test trial on the 5th day. Prior to the first trial on day 1, mice were placed beneath a box on the center of the apparatus for 15 s. After which, the box was removed and the mouse was allowed to explore the maze for a maximum time of 120 s. If the mouse found and entered the goal box, they were returned back to the cage; however if the mouse did not find and enter the box within the 120 s, they were led to the goal. Mice underwent two trials a day with a 15-min inter-trial interval. After the training phase, a 24-h probe was performed, in which the goal box was removed. Mice were placed in the middle of the maze and a 120-s time limit was allotted for exploring, in which, errors, entries (target head pokes), and latency to find the target were tallied and measured.

Tissue preparation

Mice were deeply anesthetized with sodium pentobarbital and euthanized by perfusion transcardially with 0.1 mol/L PBS, pH 7.4. Half of the brain was fixed for 48 h in 4% paraformaldehyde in 0.1 mol/L phosphate-buffered saline (pH7.4) and cryoprotected in 30% sucrose for immunohistochemical (IHC) analysis, where the other half of the brain was microdissected and the hippocampus was frozen in dry ice for biochemical