

Effects of Chronic 5-Bromo-2-Deoxyuridine Administration on Spatial Memory in the Adult Rats

Mahmoud Hosseini,¹ Abas Mohammadipour,^{*2} Azadeh Feizpour,¹ Vahid Ebrahimi²

1. Department of Physiology, Mashhad University of Medical Sciences, Mashhad, Iran
2. Department of Anatomy, Mashhad University of Medical Sciences, Mashhad, Iran

Article information	Abstract
<p>Article history: Received: 21 Feb 2012 Accepted: 22 Oct 2012 Available online: 18 Nov 2012 ZJRMS 2013; 15(5): 22-24</p> <p>Keywords: 5-Bromo-2-deoxyuridine Spatial memory Rat</p> <p>*Corresponding author at: Department of Anatomy, Faculty of medicine, University of Mashhad, Mashhad, Iran. E-mail: Mohammadia881@mums.ac.ir</p>	<p>Background: 5-Bromo-2-deoxyuridine (BrdU) has been a principal marker for mitotic cells in studies of adult neurogenesis. The method consists of a pulse injection of BrdU into the intraperitoneal cavity followed by a variable survival time allowing for tracking the divided cells and their progeny. However, such exogenous markers may produce toxic effects. Aim of this study was determined the effects of Brdu on spatial memory in the adult rat.</p> <p>Materials and Methods: 16 Wistar rats were used in this experimental study. The rats were randomly divided into 2 groups (N=8 in each group), as follows: control and Brdu (50 mg/kg). Brdu was administered intraperitoneally for 6 weeks and then animals were used for behavioral testing in the Morris water maze. The data were analyzed with repeated measure's ANOVA.</p> <p>Results: Our present findings show that there were no differences in the path length, escape latency and swim speed between control and Brdu-administrated groups.</p> <p>Conclusion: This study show that Brdu (exogenic proliferation marker) did not has side effects on spatial memory in the adult rats.</p> <p>Copyright © 2013 Zahedan University of Medical Sciences. All rights reserved.</p>

Introduction

Five-Bromo-20-deoxyuridine (BrdU) pulse labeling is commonly used for determining the day of birth of different proliferating cell populations during a determined period [1-4]. BrdU is also used in adult animals as a principal marker for mitotic cells [5]. One useful feature of BrdU is its long-term retention in divided cells and its passage to their daughter cells. This feature can be used to trace the cell lineage and cell survival [6]. BrdU is an exogenic marker and the method consists of a pulse injection of BrdU into the intraperitoneal cavity [7, 8]. Such exogenous markers may produce toxic effects. It is, however, demonstrated both in vitro [6] and in vivo that BrdU might be neurotoxic, and higher amount of BrdU can provoke various cellular abnormalities. The possibility of BrdU producing mutated cells and the consequent severe abnormalities of the developing tissues has been reported [9]. Maternal BrdU exposure, when injected into pregnant rodents, may cause exencephaly, cleft palate and limb abnormalities in the offspring [5, 10]. Moreover such recent studies indicated that Brdu administration may decreases postnatal neurogenesis in the animals [6, 11, 12]. Therefore, because of relationship between neurogenesis and memory, it is likely that BrdU changes in the memory. So in this article the effect of Brdu on spatial memory was investigated in the adult rat.

Materials and Methods

Sixteen adult male Wistar rats were used in this experiment. The experimental procedures were performed

in accordance with the animal care guidelines of the Mashhad University of Medical Sciences. The rats were housed under controlled temperature ($20\pm 2^{\circ}\text{C}$) and lighting (12 h light: 12 h dark photoperiod) conditions and permitted free access to food and water. The rats were randomly divided into 2 groups (N=8 in each group), as follows: control and Brdu (50 mg/kg).

Rats in the Brdu group received 50 mg/kg BrdU (Sigma Chemical Co. USA) intraperitoneally once a day for 6 consecutive weeks. At the same time, normal saline was injected into rats of control group [13]. After 6 weeks, the acquisition of spatial recognition memory in all animals was detected by Morris water maze.

A circular black pool (136 cm diameter, 60 cm high and 30 cm deep) was filled with water ($23-25^{\circ}\text{C}$). A circular platform (10 cm diameter, 28 cm high) was placed within the pool and was submerged approximately 2 cm below the surface of water in the center of the Northeast quadrant. Outside the maze, fixed visual cues were present at various locations around the room (i.e., computer, hardware, and posters). The animals performed four trials on each of the five consecutive days, and each trial began with the rat being placed in the pool and released facing the side wall at one of four positions (the boundaries of the four quadrants, labeled North (N), East (E), South (S), and West (W)). Morris water maze is shown in schematic form in the figure 1. Release positions were randomly predetermined. For each trial, the rat was allowed to swim until it found and remained on the platform for 20 seconds. If 60 seconds had passed

and the animal had not found the platform, it was guided to the platform by the experimenter and allowed to stay there for 20 seconds. The rat was then removed from the pool, dried and placed in its holding bin for 20 seconds. The time latency to reach the platform and the length of the swimming path were recorded by a video tracking system. Data were analyzed using the SPSS-16 for Windows. The data were analyzed statistically by repeated measures ANOVA. The significant level was set at $p < 0.05$.

Results

Elapsed time to reach the hidden platform in the control group was 16.32 ± 2.4 seconds and Brdu recipient animals spent 16.1 ± 1.88 seconds to finding the target platform. Considering the $p < 0.05$, There was no significant difference between the 2 groups (Fig. 2). The control group was 414.27 ± 63.12 centimeters over to finding the hidden platform while the Mileage to reach the target platform in the Brdu recipient group was 359.48 ± 63.12 centimeters that considering the $p < 0.05$, we don't observed significant difference between the 2 groups (Fig. 3). Also the results showed that speed of control animals and the Brdu recipient animals to reach the platform was 23.61 ± 1.057 and 23.81 ± 0.65 centimeters per second respectively that considering the $p < 0.05$, there was no significant difference between the 2 groups (Fig. 4).

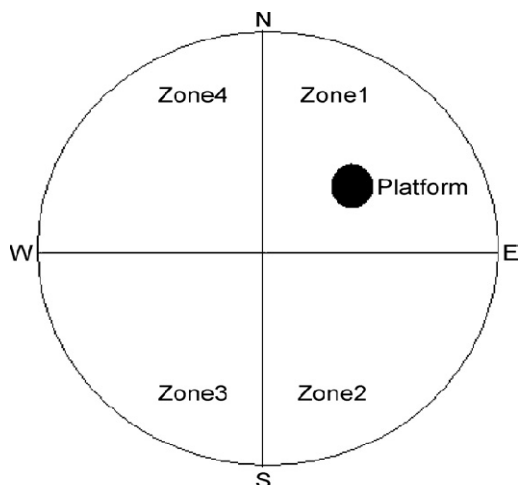


Figure 1. Schematic representation of the Morris water maze

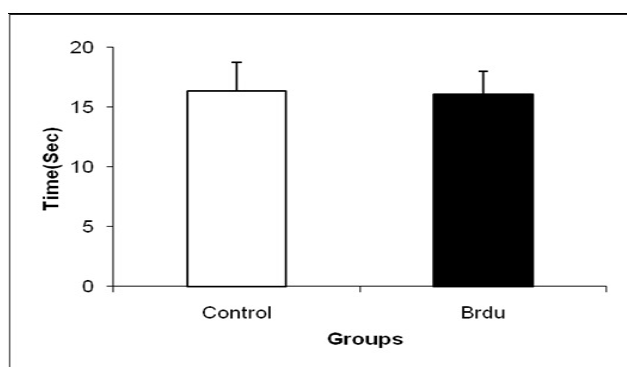


Figure 2. Comparing the time spent to find the platform

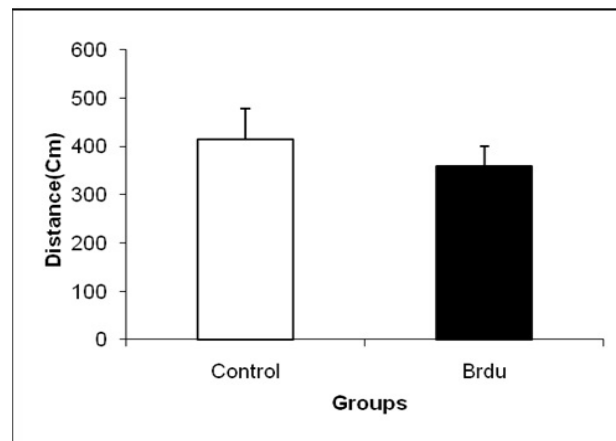


Figure 3. Comparison of distance to reach platform

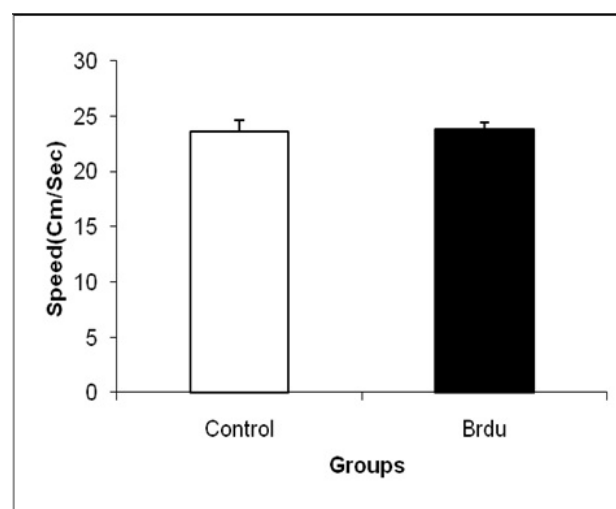


Figure 4. Comparison of speed between two groups

Discussion

Based on the results of this research, Brdu administration had no effect on memory and there was no significant difference between control and Brdu group. Results of previous studies also confirmed it. Brdu as an exogenous marker of cell proliferation widely is used in the researches. On the other hand, some previous studies reported cytotoxic effects of Brdu. Results of a previous study showed that Brdu administration decreased neurogenesis [6]. Also results of another study reported that retardation in cerebral cortex development induce by Brdu treatment in mice offspring [5]. While some studies reported that Brdu treatment had no side effect on the hippocampus. For example it has proved that Brdu administration in the adult rat didn't had any effect on hippocampal neurogenesis [10]. Moreover another study report that Brdu treatment during pregnancy had no effect on neurogenesis in the rat pups [14]. In the present study, we observed that Brdu treatment had no side effect on memory in the adult rats. So it seems that although the Brdu is an exogenous marker but use of it in the adult animals won't has significant effect on memory.

It appears that the administration of Brdu at different age periods show different effects. Based on the results of previous studies this substance had no effects on the brain of adult animals [10]. Moreover Brdu administration during embryonic period didn't show any side effect on the neurogenesis [14]. While Brdu treatment during lactation period had sever effect on neurogenesis and it decreases new born neuron in the brain [5]. As regards Brain development and hippocampal neurogenesis is mostly done in the lactation period, so Brdu treatment at this period induces the most effects on the brain [15].

Thus it is suggested that researchers use an endogenic marker of proliferation such as ki-67 instead of Brdu for studies in infants. Citing the results of this study, use of

Brdu as a cell proliferation marker in the adult animals no problem and it doesn't has side effect on the memory.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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