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Adult neurogenesis reduction by a cytostatic treatment improves spatial reversal learning in rats

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ABSTRACT

Adult neurogenesis in the dentate gyrus adds a substantial number of new functional neurons to the hippocampus network in rodents. To date, however, the function of these new granule cells remains unclear. We conducted an experiment to assess the contribution of adult neurogenesis in the dentate gyrus to acquisition and reversal learning in a task that predominantly requires generalization of a rule. Young adult male Long-Evans rats were repeatedly administered either a cytostatic temozolomide or saline for a period of four weeks (3 injections per week). Post treatment, animals were injected with bromodeoxyuridine to quantify adult neurogenesis in the dentate gyrus. For behavioral assessment we used hippocampus-dependent active place avoidance with reversal in a Carousel maze. Animals first learned to avoid a 60° sector on the rotating arena. Afterwards, sector was relocated to the opposite side of the rotating arena (reversal). The administration of temozolomide significantly improved the reversal performance compared to saline-treated rats. Our results suggest a significant, level-dependent, improvement of reversal learning in animals with reduced adult neurogenesis in hippocampus.

1. Introduction

High adult neurogenesis in the dentate gyrus (DG) has often been recognized to aid ability to discriminate between two distinct similar contexts, while generalization has been proposed to be a result of decreased adult neurogenesis (Besnard & Sahay, 2015; Kheirbek, Klemenhagen, Sahay, & Hen, 2012). Thus, capabilities to discriminate and generalize are somewhat exclusive. Ability to discriminate is beneficial in solving many of memory tests (which tests strength of memory based on discriminating two similar stimuli of which one was presented in a past). In certain situations, however, generalization is beneficial as well, for example when rules are needed to be applied to new contexts. Indeed, lately it was suggested that tasks should be evaluated based on degree of requirement for discrimination and generalization when assessing function of adult neurogenesis in hippocampus (Hersman, Rodriguez Barrera, & Fanselow, 2015). Behavioral studies indicate that this may be a reasonable direction when hypothesizing about functional role of adult neurogenesis. Conclusions of studies using contextual discrimination tests have been consistent, and agree that less adult neurogenesis means worse separation between two similar contexts (Niibori et al., 2012; Sahay et al., 2011; Tronel et al., 2012). Performance in spatial discrimination tasks is also impaired in adult neurogenesis deficient animals when the presented choices are proximal but not when far apart (Clelland et al., 2009 but see Swan et al., 2014). Unfortunately, there are not many tasks can be viewed as those requiring generalization more than discrimination. In one study, selective ablation of adult neurogenesis in knockout mice model increased generalization to novel auditory stimuli (Cushman et al., 2012). Another task which could require generalization more than discrimination is the active place avoidance in Carousel maze – the task we utilized in the experiment described here. In this task the animal avoids a directly imperceptible sector on a rotating arena. This task is not intuitive to animals, because they have to disregard cues present in the arena while using only extra-arena cues to avoid the correct to-be-avoided sector (cognitive coordination, Wesierska, Dockery, & Fenton, 2005). Importantly, precise knowledge of the sector's

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position is not necessary, because for successful avoidance it is sufficient to move in a counter-rotation direction in any safe part of the Carousel maze (more than 83% of arena). From this perspective Carousel maze reversal task can be considered to require understanding of a rule more than discrimination. In this study we assessed the contribution of neurogenesis to acquisition and reversal learning in this task. In line with hypothesis that low adult neurogenesis facilitates generalization of a rule we found normal acquisition learning and consistent improvement in reversal learning when adult neurogenesis was reduced.

2. Material and methods

2.1. Animals

For all experiments we used Long-Evans male rats from the breeding colony of the Institute of Physiology CAS. The age of animals was five weeks upon arrival – one week after the weaning (28 days). Rats were separated into cages, with each cage containing three animals. All animals were given food and water *ad libitum* and were kept on a 12/12 light/dark cycle with lights on at six am, and housed in an accredited animal room with constant temperature and humidity (22 ± 1°; 50%). Rats underwent 10 days of acclimatization and handling for 2 min each day based on an established procedure (Stuchlik, Rehakova, Rambousek, Svoboda, & Vales, 2007) before TMZ treatment begun. Rats were randomized into control and treatment groups and were labeled with a pen on their tails for identification. All animal procedures complied with the Animal Protection Act of the Czech Republic, EU directive 2010/63/EC and NIH guidelines. They were approved by Ethical Committee for proper procedures in animals and welfare of the Institute of Physiology, Czech Academy of Sciences and by Resort Committee of the Czech Academy of Sciences (Project of Experiments No. 136/2013).

2.2. Experimental design

First, we sought to find the optimal dose of temozolomide (TMZ) that would efficiently ablate the production of new neurons, but at the same time minimize unwanted side-effects. We tested the effects of TMZ at a dose of 10, 25 and 40 mg/kg body weight on adult neurogenesis, myelosuppression, and body mass. Based on these results, the second cohort of rats was treated with a dose of 25 mg/kg TMZ and tested in active place avoidance with reversal in Carousel maze. After finding that this group was superior to the control group, we repeated testing in a Carousel maze with the third cohort of rats using the lowest dose of TMZ (10 mg/kg) to avoid the possible confounding effects of the drug treatment. Experimental design is graphically illustrated in Fig. S1 (Supplementary material).

2.3. Drug treatment

To reduce adult neurogenesis, animals underwent a chronic, four-week TMZ treatment. TMZ was administered via an intragastric probe on three consecutive days every week (Tuesday-Thursday). Animals were treated between 9 and 11 am. The TMZ solution was prepared fresh from capsules (Temodal® 20 mg or 100 mg, Schering Plough, Germany) that were dissolved in acidic saline (titrated to pH 3.5 with hydrochloric acid). The TMZ solution was vortexed for two min and placed on ice for 30 min while shaking at 50 rpm on an orbital shaker to dissolve. Before application, the TMZ solution was filtered through a 0.45 μm filter to remove insoluble residues. The final concentration of TMZ was 24 mg/mL. Rats were immediately given this solution at the volume required by their respective dose category. Control animals were given equal volumes of acidic saline as a group receiving 40 mg/kg of TMZ.

The number of BrdU-positive (BrdU+) cells was used to determine the level of reduction in adult neurogenesis. On the last day of TMZ treatment, animals (cohort used to determine the correct dose and cohort treated with 10 mg/kg of TMZ and controls) were injected intraperitoneally with 50 mg/kg BrdU dissolved in saline at 40 °C. This amount has previously been shown to be sufficient to label proliferating cells (Garthe, Behr, & Kempermann, 2009).

After the TMZ treatment rats were left without intervention for three weeks to recover. After this time the animals used to test the optimal dose were sacrificed to inspect to what degree the numbers of adult-born neurons in the DG were altered by various TMZ doses. Animals used in behavioral testing were sacrificed after behavioral tests and the level of adult neurogenesis was assessed.

2.4. Control tests

All control tests were conducted at three critical time points during the procedure: before TMZ treatment (pre-TMZ), immediately after TMZ treatment (post-TMZ) and after the three-week recovery period (after recovery). Moreover, doublecortin positive cells (DCX+) were labeled to assess recovery of neurogenic niche after the recovery period.

200–450 μL of blood was gently extracted from tail by an experienced technician. Blood samples were sent for analysis to the Freston Laboratory s.r.o., Czech Republic. Counts of erythrocytes and leukocytes were measured on a veterinary hematology analyzer (Abacus Junior Vet 5, Diatron MI PLC, Hungary). Since TMZ causes myelosuppression, a reduction in the number of white blood cells in all treatment groups compared to control group was expected right after TMZ treatment (POST).

Beam walking test as described by Hamm, White-Gbadebo, Lyeth, Jenkins, and Hayes (1992) was used to assess sensorimotor deficit. Beam used here was 2 m long and 2 cm thick. Number of slips averaged from three consecutive runs was used as a main measure. To avoid inducing boredom or helplessness, animals from the same cage always finished one run before moving to the next run.

Finally, body weight was monitored as an important indicator of health because it reflects appetite and natural growth. Accordingly, animals were weighed weekly.

2.5. Immunohistochemistry

After either the recovery period (n = 30) or the Carousel maze testing (n = 40), animals were sacrificed and used to determine the level of adult neurogenesis in the DG. Animals were deeply anesthetized with ketamine/xylazine and perfused transcardially first with 0.2 M phosphate buffer and then with 4% paraformaldehyde (PFA) in phosphate buffer. Both solutions were ice-cold to constrict capillaries. Brains were removed and post-fixed in 4% PFA for 24 h. Subsequently, the brains were cryoprotected by soaking consecutively in 10%, 20% and 30% sucrose solutions in phosphate buffer. Afterwards, brains were frozen on dry ice and kept until use in a −80 °C freezer. Using a Leica 1850 cryostat, brains, tempered to −20 °C, were sectioned in 40-μm slices in the coronal plane. Slices were placed into cryoprotectant and kept at −20 °C until use. Every sixth section was used for counting BrdU+ cells.

To detect BrdU positive cells, the sections were first thoroughly washed from cryoprotectant with Tris buffered saline (TBS). Endogenous peroxidases were deactivated by a 10 min treatment with 3% hydrogen peroxide and 10% methanol in TBS. After thorough washing in TBS and 0.9% NaCl, the DNA was denatured for 30 min with 2.5 N HCl at 37 °C. Slices were washed in several changes of phosphate buffered saline (PBS) and then incubated in