Neuroscience Letters 703 (2019) 96–98

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

BrdU-induced hyperlocomotion in the stroked rat

Jessica Cooperrider[a,1], Hugh H. Chan[a,1], John T. Gale[a,b], Hyun-Joo Park[a], Kenneth B. Baker[a,b], Andre G. Machado[a,b,*]

[a] Department of Neurosciences, Lerner Research Institute, Cleveland Clinic, Cleveland, OH 44195, United States
[b] Center for Neurological Restoration, Department of Neurosurgery, Neurological Institute, Cleveland Clinic, Cleveland, OH 44195, United States

ARTICLE INFO

Keywords:
BrdU
Bromodeoxyuridine
Neurorehabilitation
Neurogenesis
Ischemia

ABSTRACT

5-bromo-2′-dexoyuridine (BrdU) is often used in neuroscience research as a marker of newly-divided cells. However, several studies suggest that BrdU can produce unwanted side effects, including changes in animal behavior and cellular function. In this study, we investigated the effect of BrdU injections on locomotor behavior in a rodent model of ischemic stroke. Ischemic strokes were induced in adult rats, and 50 mg/kg BrdU was intraperitoneally injected over 5 days beginning 2 weeks post-stroke, while control animals received vehicle. Locomotor activity was evaluated by videotaping the rats in their home cages for 30 min, beginning one hour after BrdU injection. BrdU-injected rats showed a nearly three-fold increase in locomotor activity compared to control animals. These findings suggest that BrdU induces a hyperlocomotor effect in rats following brain injury, pointing to the need for caution when interpreting behavioral results in such studies.

1. Introduction

5-Bromo-2′-deoxyuridine (BrdU), a thymidine analogue, is commonly used to label proliferating cells and is considered the "gold standard" method for identifying neurogenesis. BrdU also has known neurotoxic properties resulting in, among many other effects, dilation of the lateral ventricles, impaired development of the cortical plate, and decreased brain weight in the offspring of injected dams [1–3]. It is well established that prenatal exposure to BrdU induces hyperactivity [1,4,5], with the offspring of BrdU-injected pregnant dams even pursued as a putative model of attention deficit hyperactivity disorder (ADHD) [6,7]. It has not been demonstrated, however, that BrdU promotes hyperlocomotion when administered to postnatal animals.

Our group has shown that chronic electrical stimulation of the lateral cerebellar nucleus of the rat following cerebral ischemia can promote substantial motor recovery [8,9]. As part of our ongoing exploration into the mechanism of this therapy, we injected animals with BrdU in order to examine the potential mechanistic role of neurogenesis in motor recovery. We observed an abnormal increase in activity, including increased jumping and ambulation in multiple BrdU-injected animals (unpublished observation). Due to the ubiquity of BrdU use in post-injury mechanistic studies [10–12], we thought it critical to quantify these anecdota to determine whether BrdU directly influences motor behavior in adult, post-stroke rodents. We hypothesized that the plastic, post-stroke brain may behave in a similar way in response to BrdU as the young, maturing brain, resulting in a hyperactive state. Here, we characterize changes in the locomotion of rats injected with BrdU two weeks post-stroke.

2. Materials and methods

2.1. Animals

Eight male Long Evans rats weighing 200–224 g at study onset were individually housed in custom-made caging on a 12-h light/dark cycle with free access to food and water. Behavioral analysis was performed during the dark phase of the light cycle under controlled red lighting. All animal work performed was approved by the Institutional Animal Care and Use Committee of the Cleveland Clinic.

2.2. Surgical procedure for endothelin 1-induced stroke

Focal ischemia was induced as detailed previously [8,9]. Briefly, rats were anesthetized with an intramuscular administration of 50 mg/kg ketamine (Fort Dodge, IA, USA) and 0.5 mg/kg dexmedetomidine (Orion, Finland) and fixed in a stereotaxic frame (David Kopf Instruments, CA, USA). Burr holes were drilled over the left sensorimotor cortex, and ischemia induced by intracortical injection (0.5 µl/min) of

* Corresponding author at: Cleveland Clinic, Neurological Institute/T13, 9500 Euclid Avenue, Cleveland, OH 44195, United States.
E-mail address: machada@ccf.org (A.G. Machado).
[1] J.C. and H.H.C. contributed equally to this work.

https://doi.org/10.1016/j.neulet.2019.03.007
Received 1 November 2018; Received in revised form 21 February 2019; Accepted 5 March 2019
Available online 07 March 2019
0304-3940/

800 pmol/2 µl endothelin 1 (ET-1, 05-23-3800, Millipore, MA, USA) at each of the following coordinates in relation to bregma [9]: (1) AP: −1.0 mm, ML: +2.5 mm, DV: −2.3 mm; (2) AP: +1.0 mm, ML: +2.5 mm, DV: −2.3 mm; and (3) AP: +3.0 mm, ML: +2.5 mm, DV: −2.3 mm; (4) AP: −1.0 mm, ML: +3.5 mm, DV: −2.3 mm; (5) AP: +1.0 mm, ML: +3.5 mm, DV: −2.3 mm; and (6) AP: +3.0 mm, ML: +3.5 mm, DV: −2.3 mm. The burr holes from ET-1 injection were covered with a patch of cellulose paper (Data Sciences International, MN, USA) that was sealed with tissue adhesive (Vetbond; 3 M, MN, USA). Medetomidine anesthesia was reversed with atipamezole (1 mg/kg, s.c., Orion, Finland), followed by buprenorphine administration for pain management. Animals were monitored postoperatively and received routine analgesic management with buprenorphine (0.05 mg/kg, s.c., Reckitt Benckiser, VA, USA) for two days. The animals were allowed to recover with food and water *ad libitum*.

2.3. Administration of BrdU

Following a two-week recovery period from cortical ischemia, four male rats received a single dose of 50 mg/kg BrdU (100171, MP biomedicals, OH, USA) intraperitoneally for five consecutive days (BrdU+). 50 mg/kg BrdU was chosen because it is a commonly used dose of BrdU that provides adequate cell labeling but is associated with minimal toxicity. BrdU was administered for five consecutive days to replicate the conditions in which our group first noticed that BrdU may be inducing hyperactivity in stroked rats. An additional four male control animals received intraperitoneal injections of an equivalent volume of vehicle (saline, BrdU−).

2.4. Locomotor activity

The locomotor activity of each rat after BrdU or vehicle administration was videotaped using an analog camera installed at the top of each home cage. We chose to videotape behavior in the home cage to evaluate whether behavioral abnormalities are present even when animals are habituated to their environment. Videotaping began one hour after each daily injection and concluded following 30 min of filming. The distance travelled by each rat during that period was calculated using the video tracking software, EthoVision (Noldus, MA, USA), by experimenters blinded to group assignment.

2.5. Histology

Rats were deeply anesthetized by 50 mg/ml Nembutal and then decapitated. Brains were fixed in 4% phosphate buffered paraformaldehyde (EMS, PA, USA) for seven days. After cryoprotection in 30% sucrose/PBS, brains were snap-frozen and stored at −80 °C until slicing by cryostat. 30µm sections of cerebral cortex were mounted on polysine-coated slides. Sections containing the lesion were Nissl stained and stroke volume was calculated as previously described [13].

2.6. Statistics

Between-group comparisons were analyzed with Student's *t*-tests (GraphPad Prism, CA, USA). Statistical results were considered significant when $p < 0.05$, as annotated in the figures.

3. Results

3.1. Stroke size

Intracortical injection of ET-1 produced an ischemic lesion at the motor cortex in both BrdU− (12.99 ± 0.53 mm^3 [mean ± SEM]) and BrdU+ (12.52 ± 0.58 mm^3) groups. A Student's *t*-test did not demonstrate a significant difference between groups ($t(6) = -0.60$, $p = 0.57$). Fig. 1 presents a pooled illustration of stroke volume location and extent.

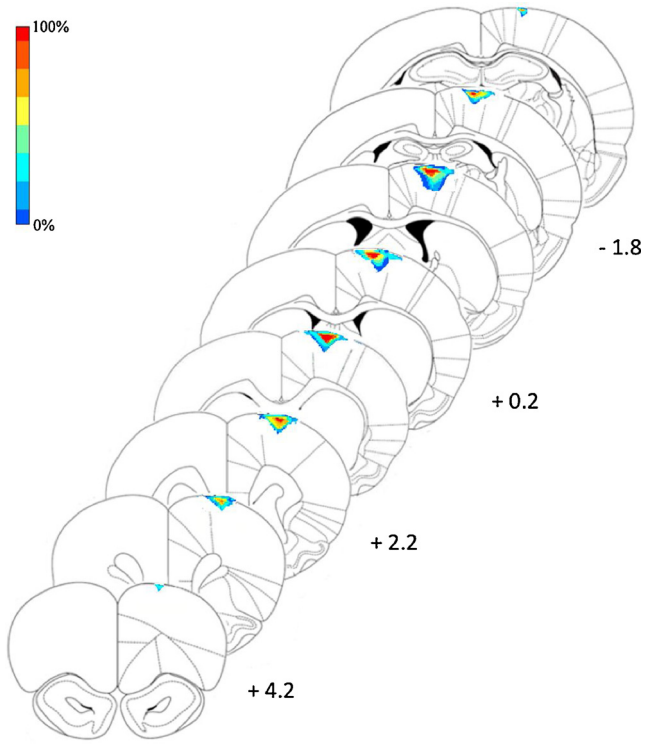

Fig. 1. Histological examination of infarct location and volume. Infarct location and volume are overlaid on coronal sections from the Paxinos and Watson Atlas [14]. Infarcts spanned from approximately 4.2 mm anterior to −2.8 mm posterior to bregma. Color coding represents the percentage of animals with infarcted tissue present at that pixel.

3.2. Effect of BrdU on the locomotor activity of the stroked rat

A 5-day administration of BrdU two weeks after stroke induction was associated with a significant increase in distance traveled over 30 min (424.7 cm ± 55.79 [mean ± SEM], Fig. 2) as compared to animals injected with vehicle alone (167.9 cm ± 42.61, $t(6) = 3.66$, $p = 0.01$, Fig. 2).

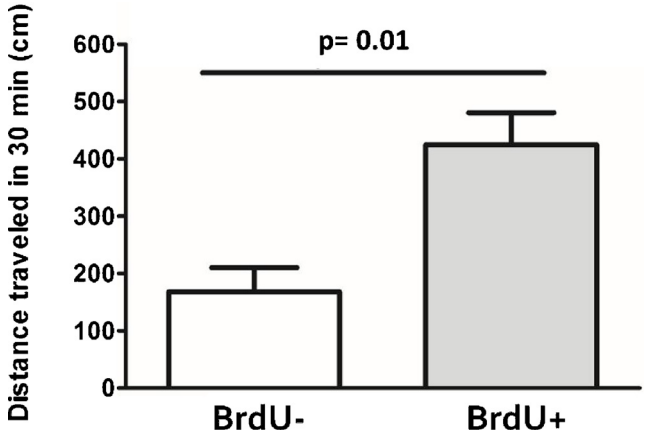

Fig. 2. Locomotion in the home cage following BrdU or vehicle injection. Animals were videotaped and the distance traveled in 30 min quantified offline by raters blinded to group. A Student's *t*-test was used to compare the distance traveled between groups.

4. Discussion

The results presented here reveal an association between administration of BrdU and increased motor behavior, consistent with previous studies [1,4,5]. To our knowledge, this is the first study to identify a link between administration of BrdU in adult animals and acute behavioral changes using a commonly administered dose (50 mg/kg) that is otherwise thought to have minimal side effects [15]. Overall, the data support our hypothesis that the developing brain and the post-ischemic brain are similarly susceptible to the cytotoxic effects of BrdU, resulting in a relative hyperactivity. BrdU has been shown to decrease the number of GABAergic neurons in somatosensory cortex when given postnatally at P0 through P5 or P11 mice, with cortical slices of these animals also displaying increased excitability [16]. This increased cortical excitability provides a mechanism through which hyperlocomotion could occur following BrdU administration. Dopamine has also been suggested to play a role in the mechanism of BrdU-induced hyperactivity, with decreased dopamine present in the striatum of prenatally injected animals [6]. Of note, in this study, there was no difference in lesion size between the BrdU-injected and vehicle-injected groups, indicating that BrdU administered at two weeks post-stroke did not affect the already-established stroke volume.

In the future, the behavior of age-matched non-stroked animals treated with BrdU should be evaluated in order to better interpret the present results. We chose to evaluate post-stroke animals in this study because BrdU is commonly used in post-stroke mechanistic studies, and we thought that there should be a primary focus on understanding the effects of BrdU in this condition. Furthermore, Kolb et al. [2] have demonstrated that the administration of BrdU postnatally to naive animals at P10 does not disrupt the performance of a skilled reaching task, although hyperactivity resultant from postnatal injection has not yet been evaluated. We also acknowledge that the results of this study are only generalizable to the post-stroke state, and that the effects of BrdU will need to be examined in various models of CNS injury in order to draw any overarching conclusions about the effects of BrdU on motor behavior. An investigation of the timeline of administration of BrdU post-stroke or post–injury could also provide insight into the mechanism of hyperactivity, particularly with regard to whether a requisite sensitive period is necessary for the behavioral changes seen here. Additionally, unlike a previous study in which BrdU was given prenatally with animals demonstrating hyperlocomotion in the open field but not in their home environment [7], our postnatally-administered rats showed hyperactivity in their home cages. Future studies with a more in-depth behavioral analysis may demonstrate whether this behavioral modification is generalizable to the open field setting. Furthermore, we cannot conclusively say that BrdU-treated animals displayed hyperactivity compared to naïve animals, as this study did not utilize non-stroked animals. However, we can conclude that BrdU induced a nearly threefold relative hyperactivity when compared to non-BrdU-treated stroked animals.

Although this study is preliminary in nature and scope, we believe that due to the pervasiveness of BrdU-administered studies, further investigation is required, and caution must be taken when interpreting BrdU-related behavioral results, particularly after brain injury.

Declaration of interest

Dr. Machado has distribution rights related to intellectual property in Enspire DBS and Cardionomics and is a consultant for St. Jude Medical.

Acknowledgement

The work was funded by National Institutes of Health Grant R01 HD061363.

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