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A reproducible Endothelin-1 model of forelimb motor cortex stroke in the mouse

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HIGHLIGHTS

- Intra-cortical injections of Endothelin-1 generate a reproducible ischemic injury.
- Infarcts targeted to the anterior FMC result in consistent behavioral deficits.
- Paw-dragging is a novel analysis of the mouse cylinder test.
- Paw-dragging is a highly sensitive measure of forelimb motor cortex damage.

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ABSTRACT

Background: Despite the availability of numerous transgenic mouse lines to study the role of individual genes in promoting neural repair following stroke, few studies have availed of this technology, primarily due to the lack of a reproducible ischemic injury model in the mouse. Intracortical injections of Endothelin-1 (ET1) a potent vasoconstrictive agent, reliably produces focal infarcts with concomitant behavioral deficits in rats. In contrast, ET1 infarcts in mice are significantly smaller and do not generate consistent behavioral deficits.
New method: We have modified the ET1 ischemia model to target the anterior forelimb motor cortex (aFMC) and show that this generates a reproducible focal ischemic injury in mice with consistent behavioral deficits. Furthermore, we have developed a novel analysis of the cylinder test by quantifying paw-dragging behavior.
Results: ET1 injections which damage deep layer neurons in the aFMC generate reproducible deficits on the staircase test. Cylinder test analysis showed no forelimb asymmetry post-injection; however, we observed a novel paw-dragging behavior in mice which is a positive sign of damage to the FMC.
Comparison with existing methods: Previous ET1 studies have demonstrated inconsistent behavioral deficits; however, targeting ET1 injections to the aFMC reliably results in staircase deficits. We show that analysis of paw-dragging behavior in the cylinder test is a more sensitive measure of damage to the FMC than the classical forelimb asymmetry analysis.
Conclusions: We have developed a focal ischemic injury model in the mouse that results in reproducible behavioral deficits and can be used to test future regenerative therapies.

1. Introduction

Neural stem cells in the adult brain have the capacity to generate neurons and glia and thereby hold tremendous potential for neural repair following brain injury such as stroke. Despite the development of numerous transgenic mouse lines to study the role of individual genes and genetic pathways in promoting neural

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repair mechanisms following stroke, few studies have availed of the transgenic technology, primarily due to the lack of a reproducible ischemic injury model in the mouse.

A number of ischemic injury models currently used in mice lack relevance to stroke injury in humans. The intraluminal suture model of middle cerebral artery occlusion (MCAO) in mice results in massive infarcts that range in size from 21 to 45% of the affected hemisphere (Carmichael, 2005). This extent of damage lacks clinical relevance because comparable stroke damage in humans is non-treatable (Carmichael, 2005). The majority of treatable strokes in humans range in size from 4.5 to 14% of the affected hemisphere (Carmichael, 2005). The photothrombosis model results in permanent ischemia with no reperfusion and the injury profile shows both vasogenic edema and cytotoxic edema whereas in humans only cytotoxic edema is observed (Carmichael, 2005; Lipsanen and Jolkkonen, 2011). Therefore there exists a need for a reproducible focal ischemic injury model in mice that has relevance to the clinic in which to study mechanisms of neural repair.

There are reproducible focal ischemic injury models currently in use in rats however; these models produce more variable responses in mice. For instance, intracortical or subcortical application of the vasoconstrictive peptide, Endothelin-1 (ET1) in the rat reliably produces focal infarcts with quantifiable behavioral deficits (Fuxe et al., 1992; Windle et al., 2006). In contrast, ET1 infarcts in mice are significantly smaller and most behavioral deficits resolve within 3–5 days post-injury (Tennant and Jones, 2009; Wang et al., 2007). However, previous studies used low concentrations of ET1 and did not have access to the recently mapped mouse forelimb motor cortex (Tennant et al., 2011). By taking into consideration the distinctions between mice and rats including a lower sensitivity to ET1 (Horie et al., 2008; Wiley and Davenport, 2004) and differences in the map of the forelimb motor cortex (FMC) (Tennant et al., 2011) and performance in behavioral tests, we sought to determine whether ET1 could be used to create an efficient and reproducible ischemic injury model in the mouse.

The goal of this study therefore was to generate a reproducible focal ischemic injury model in the mouse that resulted in persistent behavioral deficits that could be used to test future therapeutic strategies. Taking into consideration the recommendations set out by the Stroke Therapy Academic Industry Roundtable (STAIR) (STAIR, 1999), we identified four essential criteria that the ideal model must meet. First, the model must generate a reproducible lesion to minimize inter-animal variability such that accurate assessments of neuroprotection and regeneration can be measured between experimental and control-treated animals. Neural regeneration studies need to demonstrate not only tissue regeneration, but also recovery of function. Therefore, the second criterion is that the ischemic injury must result in a specific and reproducible behavioral deficit that is persistent. Although a battery of behavioral tests have been developed to assess functional deficits in rats post-stroke (Clarke et al., 2007; Montoya et al., 1991; Schallert et al., 2000; Windle et al., 2006), their sensitivity to assess functional deficits following a small focal ischemic injury in the mouse remains to be tested. Our third criterion is that the behavioral deficit should correlate with the extent of damage. Lastly, in order to assess the regenerative response of endogenous neural precursor cells (NPCs) within the brain, the fourth criterion is the injury must generate a focal infarct that does not damage the endogenous NPC population within the subventricular zone (SVZ).

Here we show that ET1 injections targeted to the FMC generate a reproducible focal infarct with measurable behavioral deficits and a regenerative response from NPCs thereby meeting all four of our criteria for an ideal ischemic injury model.

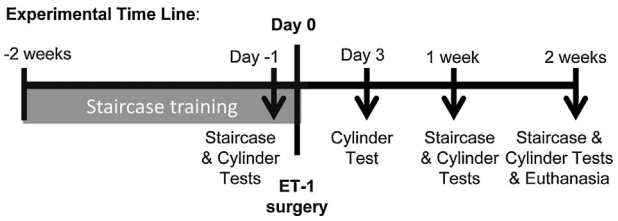

Fig. 1. Experimental timeline.

2. Methods

2.1. Mice

Adult male FVBN mice (65) between 2 and 4 months were used in this study. Mice were housed on a 12:12 h reverse light-dark cycle (lights off at 8:00 am) and given standard rodent chow and water ad libitum except where otherwise indicated. All behavior training and testing was performed during the dark cycle. All experiments were approved by Memorial University of Newfoundland's Animal Care Ethics Committee according to the guidelines of the Canadian Council on Animal Care.

2.2. Experimental setup

Mice were randomly sorted into 4 groups: (i) 2-injections of saline ($n = 15$), (ii) 2-injections of ET1 ($n = 19$), (iii) 3-injections of saline ($n = 15$), and (iv) 3-injections of ET1 ($n = 16$). Injection sites targeting the FMC are indicated by symbols in Fig. 3B. The circle and triangle symbols indicate the 2-injection sites, while the circle and plus symbols indicate the 3-injection sites. The experimental timeline is shown in Fig. 1. Mice were trained on the mouse staircase test twice per day with a 3h inter-trial interval for a minimum of two weeks prior to surgery. Staircase testing was performed twice per day over two days with the average of four staircase results recorded for each time point. Staircase testing time points included the two days before surgery = 'before', days 6 and 7 post-surgery = '1 week' and days 13 and 14 post-surgery = '2 weeks'. Mice were tested on the cylinder once a day on the day before surgery = 'before' and on days 3, 7 and 14 post-surgery. At 14 days post-surgery mice were euthanized and histology was performed to assess infarct size and location.

2.3. Surgery

Mice were anaesthetized with Isoflurane (Aerrane, Baxter 02225875) mixed with oxygen using an isoflurane vaporizer (Harvard Apparatus, 340471). At the start of surgery, Buprenorphine (0.02 mg/kg, s.c.) was injected subcutaneously as an analgesic. Injections of saline or ET1 into the mouse cortex were performed with a mouse stereotaxic instrument (David Kopf Instruments, Tujunga, CA, USA, 308019R) and all injection sites were measured in relation to bregma. For the 2-injection group, coordinates were as follows: (i) 0.0 anterior–posterior (AP), +1.50 medial–lateral (ML), −1.2 dorsal–ventral (DV) and (ii) 0.0 AP, +1.75 ML, −1.2 DV. For the 3-injection group coordinates were as follows: (i) +0.4 AP, +1.6 ML, −1.2 DV, (ii) +0.2 AP, +1.35 ML, −1.2 DV, and (iii) 0.0 AP, +1.75 ML, −1.2 DV. A hole was bored into the skull at each coordinate with an Ideal Microdrill (Cellpoint Scientific, Gaithersburg, MD, USA, 67-1000). One μl of ET1 (2 μg/μl) was injected over a 10 min period at each coordinate using a pulled glass pipette attached to a 5 μl Hamilton syringe (Hamilton Co, Reno, NV, USA, 7633-01). A syringe pump (Fusion 100, Chemyx Co, Stafford, TX, USA) was used to control flow rate at 0.1 μl/min. Following injection, the pipette was