Journal of Neuroscience Methods 239 (2015) 100–107

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

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

Basic Neuroscience

Whole-scale neurobehavioral assessments of photothrombotic ischemia in freely moving mice

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HIGHLIGHTS

- Photothrombosis was induced in freely moving mice.
- Neurobehavioral assessments of focal ischemia in a whole process.
- Motor capacity was detected throughout the development of motor cortex ischemia.

ARTICLE INFO

Article history:
Received 19 February 2014
Received in revised form 8 October 2014
Accepted 9 October 2014
Available online 18 October 2014

Keywords:
Freely moving mice
Neurobehavioral assessments
Nimodipine
Photothrombotic ischemia

ABSTRACT

Background: Neurobehavioral assessments have been considered as an essential component of preclinical research in ischemic stroke. However, real-time neurobehavioral evaluation is seldom applied during ischemia induction as it is usually accompanied with anesthesia.
New method: We induced photothrombosis in freely moving mice after one-week recovery from cannula implantation surgeries. After rose bengal (RB) injection (100 mg/kg, i.p.), photothrombosis was induced in freely moving mice by 473 nm laser irradiation through the cannulas implanted into unilateral primary motor cortex beforehand. Mice received nimodipine (15 mg/kg, i.p.), a widely used anti-ischemic agent, or vehicle before irradiation. Motor coordination and equilibrium were evaluated by rotarod and rung walk tests throughout the whole process of ischemia. Endurance capacity was assessed by treadmill at 1 day and 7 days after irradiation. Mice were decapitated at different time points post irradiation for TTC (2,3,5-triphenyltetrazolium chloride) staining.
Results: Consistent with the results of TTC staining, motor deficits firstly occurred at 15-min post irradiation and aggravated 1-day later, while the capacity improved 3-days later and partially recovered 7-days post irradiation. And, the recovery process was accelerated by nimodipine application.
Comparison with existing methods: This method established a precise linkage between focal brain ischemia development and neurobehavioral deficits throughout a full scale of photothrombosis, which avoided the confounding factors of anesthetics and surgeries on neurobehavioral assessments, as infarct was induced in freely moving mice.
Conclusions: This method with high temporal and spatial resolution will be an optimal model for neurobehavioral evaluation in preclinical anti-ischemic drug screening.

1. Introduction

Ischemic stroke can cause devastating brain damage and functional deficits such as motor disability (Carmichael, 2005). Deficit severity, which determines final functional outcome, changes quickly within limited time window of several hours after onset of ischemia (Biller et al., 1990; Saver and Altman, 2012). Thus, early intervention will be the most effective and reasonable therapeutic strategy (Oyagi and Hara, 2012; Peng et al., 2013). To evaluate the effectiveness of early intervention on functional deficits and go

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http://dx.doi.org/10.1016/j.jneumeth.2014.10.004
0165-0270/

beyond merely histological assessments in preclinical research, it is necessary to develop time-controlled and sensitive behavior analyses (Balkaya et al., 2013; Dirnagl, 2010; Papadopoulos et al., 2009). However, behavioral evaluation post stroke is relatively limited on existing paradigms. It is difficult to scale the full process of functional deficits development especially at early phase post stroke since the adverse effect of anesthetics and surgical injuries which will lead to neurobehavioral variation (Veizovic et al., 2001).

Photothrombosis induces focal ischemia in restricted brain regions by laser irradiation after injection of RB (rose bengal), a potent photosensitizing dye (Watson et al., 1985). Photo-activated RB assembles platelets to form the clot in vascular endothelium. Blood flow is reduced or even blocked after that and ischemia insult occurs. Photothrombosis, which is highly reproducible with respect to the infarct location and size, will be an optimal focal ischemia model to study behavioral alterations with high spatial resolution (Kuroiwa et al., 2009; Lee et al., 2007; Nowicka et al., 2008). Moreover, consecutive rotarod tests can accelerate recovery after photothrombosis and offer advantages to investigate a full pathologic process of ischemia (Watson et al., 1985; Dirnagl, 2010; Wang et al., 2013). However, anesthetized rodents were used in most photothrombotic ischemia models (Dirnagl, 2010; Kuroiwa et al., 2009). Adverse reactions of anesthetic and surgeries make it impossible to detect early behavioral alterations with reliable results. Besides, the immunoreactions following surgeries also cannot be excluded in the anesthetized paradigms. In this sense, a new method to induce photothrombosis in freely moving animals which can avoid the confounding variables of anesthetics and surgeries on neurobehavioral assessment is in great need.

We ameliorated photothrombosis model by implanting cannulas into targeted brain regions beforehand and therefore separated focal ischemia induction from the effect of anesthetics and surgery recovery. Recovering, mice were subjected to photothrombosis. Thus, the functional behaviors can be detected at any time point throughout the whole process.

Primary motor cortex is vulnerable to ischemic attack and motor disability is one of the most common sequels of ischemic stroke (Riahi et al., 1998). Here, we aimed to assess motor deficits at different time points after unilateral primary motor cortex ischemia while leaving the contralateral side intact as control in freely moving mice. Motor coordination and equilibrium in rotarod or rung walk tests, endurance capacity in treadmill were examined from the onset till 7 days post irradiation. Moreover, we applied nimodipine, a potential therapeutic agent widely used in histological studies (Germano et al., 1987), to detect the advantages of our paradigm in evaluating the effects of anti-ischemic drug on neurobehaviors recovery.

2. Materials and methods

2.1. Animals

Adult male Kunming (KM) mice (20–25 g, 8–10 weeks; obtained from Animal House Center, Kunming Medical College, Kunming, China, $n = 74$) were group-housed in a thermoregulated environment with relative humidity $50 \pm 10\%$. Mice were maintained on 12-h light/dark cycle with free access to food and water. Animal care and experimental protocols were approved by the animal ethics committee of Kunming Institute of Zoology, Chinese Academy of Sciences.

2.2. Cannula implantation

All mice were anesthetized by pentobarbital (80 mg/kg, 8 mg/ml in saline, i.p.) and ventilated with 95% oxygen and 5% carbon dioxide through a face mask until they woke up. The scalp was displaced from the cranium for a one-centimeter midline incision. Mice were (Dietrich et al., 1999) (RWD, Life Science Co. Ltd., Shenzhen, China) and three holes were drilled for the anchor screws to fix dental cement. Cranium was drilled above unilateral primary motor cortex. Guide cannula (22 gauges) was lowered into unilateral primary motor cortex (M1, AP: −1 mm from bregma; ML: ±1.75 mm; DV: 0.2–0.3 mm from the external scull surface) with the cap screwed (Li et al., 2008). Mice were housed within their homecage for 7-day recovery, and were accommodated in laboratory one day before neurobehavioral tests.

2.3. Photothrombosis in freely moving mice

To establish an effective photothrombosis protocol in freely moving mice, three independent groups of mice received 30 min laser irradiation (473 nm or 593 nm, Biogene, Beijing) or LED (565 nm, Thorlabs) 1 h after RB injection (100 mg/kg, 10 mg/ml in saline, i.p., Sigma, Shanghai). Optic fibers protruded 0.1 mm beyond the end of guide cannulas (0.3–0.4 mm from the external scull surface). TTC staining was used to measure the effect of different wavelengths on infarction 24 h later.

To detect motor deficits during the full-scale of photothrombosis, infarct was induced in freely moving mice during FSRR (fixed-speed rotarod test). After 2 days of baseline tests, all mice received RB injection (100 mg/kg, 10 mg/ml in saline, i.p., Sigma, Shanghai). 1 h later, mice were placed on the rod and received 473 nm laser irradiation (15–22 mW) via optical fibers (200 μm core diameter, Thorlabs) embedded in guide cannulas for the first 30 min of FSRR. Nimodipine (15 mg/kg, 1.5 mg/ml in vehicle, i.p., Sigma, Shanghai) or vehicle (10 ml/kg, i.p.) were injected 10 min before irradiation.

2.4. Rotarod tests

After recovery from surgeries, mice were placed onto motorized rod (LE8500, Panlab, Spain) rotating at 4 rpm for three consecutive trials (5 min per trial) every day before rotarod tests for adaptation. For accelerating rotarod test (ARR), the speed accelerated from 4 to 40 rpm within 30 s (Buitrago et al., 2004; Jones and Roberts, 1968; Jueptner et al., 1997; Rustay et al., 2003; Shiotsuki et al., 2010; Wang et al., 2013). The latency and velocity at which each mouse fell off the rod were recorded and the results of three consecutive trials was averaged as the final result. Mice were put back to homecage again for at least 3 h after ARR. Then, mice were placed onto the rod rotating at constant speed of 4 rpm for fixed-speed rotarod test (FSRR). The longest latency to fall among three trials was noted within maximum 2-h window (Beltran et al., 2010; Farr et al., 2006; Rustay et al., 2003). Mice sequentially underwent rung walk, rotarod adaptation, ARR, FSRR every day throughout the whole process of 10 consecutive days. To examine the real-time effects of photothrombosis, ARR was carried out after FSRR on the day of photothrombosis induction.

2.5. Rung walk tests

The rung walk apparatus was composed of five Plexiglas walls and a grid (31 plastic bars with a diameter of 0.4 cm). Spaces between rungs were different, ranging from 0.4 to 1 cm. The whole apparatus was put atop 2 standard mice cages. Every day before rotarod tests, mice underwent rung walk tests till they had finished 100 steps in the apparatus. Step missing errors (straightway and total) and time spend were recorded (Beltran et al., 2010; Farr et al., 2006; Hines and Haydon, 2013; Papadopoulos et al., 2009).