Behavioural Brain Research 336 (2018) 250–255

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

# Applicability of the grip strength and automated von Frey tactile sensitivity tests in the mouse photothrombotic model of stroke

Faisal F. Alamri[a], Abdullah Al Shoyaib[a], Abbie Biggers[a], Srinidhi Jayaraman[a], Josée Guindon[b], Vardan T. Karamyan[a,c,*]

[a] Department of Pharmaceutical Sciences, School of Pharmacy, TTUHSC, United States
[b] Department of Pharmacology and Neuroscience, School of Medicine, TTUHSC, United States
[c] Center for Blood Brain Barrier Research, School of Pharmacy, TTUHSC, United States

ARTICLE INFO

*Keywords:*
Photothrombosis
Stroke recovery
Sensorimotor test
Mechanical sensitivity
Digital electronic von Frey
Anesthesiometer

ABSTRACT

Improvement of impaired neurological function(s) is a primary endpoint in experimental stroke recovery studies, making the choice and nature of the functional tests crucial for proper execution and interpretation of such studies. Currently, there are a limited number of neurological tests which reliably evaluate functional deficit in mice over a long period of time after stroke. In this study, we evaluated the applicability of forepaw grip strength and automated von Frey tactile sensitivity tests to assess forelimb dysfunction in mice following photothrombosis in the sensorimotor cortex, and compared them with two well-established tests, grid-walking and cylinder, for up to 21 days after stroke. Our results indicate that the length of time required to conduct the two new tests is comparable to that of the grid-walking and cylinder tests, however the data from the new tests is obtained and ready for analysis upon completion of the testing session. In addition, our observations indicate that the automated von Frey test detected substantial and sustained deficit in the withdrawal threshold of the mice on all evaluation days after stroke, whereas the forepaw grip strength test was only marginally sensitive to document functional impairment. Our data demonstrate that the automated von Frey tactile sensitivity test is a time efficient and sensitive method which can be used together with other established tests to evaluate long-term functional outcome in the mouse photothrombotic stroke model.

## 1. Introduction

Stroke is a chronically disabling and progressive disease constituting the leading cause of adult disability. Improvement of functional recovery in disabled stroke survivors is a greatly important but currently unmet therapeutic modality, requiring both pre-clinical and clinical studies to identify safe and effective therapies. Improvement of impaired neurological function(s) is the primary endpoint in experimental and proof-of-concept therapeutic preclinical stroke recovery studies, making the choice and nature of the functional tests crucial for proper execution and interpretation of such studies. Numerous tests have been developed to assess neurological deficit and outcome in animal stroke models by evaluating motor, somatosensory, cognitive or other functions [1–6]. Notably, not all tests are suitable for every stroke model because different stroke models mimic different clinical forms of stroke and therefore result in different neurologic dysfunctions [5,7]. In addition, while mice have become the primary experimental animal in stroke research and many functional tests have been adapted from the rat, testing methods and principles vary between these two species and make comparison of the test results challenging [3,4].

Two of the main mouse ischemic stroke models used in experimental recovery studies are the photothrombotic and the endothelin-1 models [8–10], of which the former model primarily results in permanent interruption of the blood flow in the target area of the brain, whereas in the latter model there is a reperfusion component following the ischemia. Both models have been central in various studies focusing on understanding of the basic biology of the brain recovery following stroke [8,11–17], identification and validation of key molecular targets involved in post-stroke recovery [18,19] and testing of various therapeutic approaches for stroke recovery [20–24]. Two of the main functional tests used to monitor sensorimotor function in these stroke models are the grid-walking (also referred to as foot-fault) and the cylinder (also referred to as spontaneous forelimb use task) tests which have been used by multiple laboratories in recent years. Both tests are very sensitive to track functional impairment weeks to months following the stroke injury [18,20,25] and allow assessment of

* Corresponding author at: Department of Pharmaceutical Sciences and Center for Blood Brain Barrier Research, School of Pharmacy, TTUHSC, 1300 Coulter Street, Amarillo, TX, 79106, United States.
*E-mail address:* vardan.karamyan@ttuhsc.edu (V.T. Karamyan).

http://dx.doi.org/10.1016/j.bbr.2017.09.008
Received 11 August 2017; Received in revised form 29 August 2017; Accepted 4 September 2017
Available online 08 September 2017
0166-4328/ 

pharmacological and rehabilitative interventions for functional recovery [20–24]. However, these tests require massive amount of time to obtain data which involves very slow, at times frame-by-frame, viewing of the video recordings to properly document impaired function of the animals. The latter is a shortcoming and substantially affects timely completion of experimental studies. To address this drawback, in this study we evaluated the applicability of forepaw grip strength and automated von Frey tactile sensitivity tests to assess forelimb dysfunction following stroke in the mouse photothrombotic model and compared them with the grid-walking and the cylinder tests for up to 21 days after ischemic injury. While the grip strength and von Frey tests have been used in some experimental stroke studies, most of them were not designed to directly evaluate these tests with other established tests in the chronic phase of stroke, they lacked important methodological details and none was conducted in the mouse photothrombotic model, which is the expert-recommended model of choice for preclinical stoke recovery studies in mice [26]. The results of the current study indicate that while the length of time required to conduct the two new tests is comparable to that of the grid-walking and cylinder tests, the data from the new tests is obtained and ready for analysis upon completion of the testing session. In regard to sensitivity of the new tests to detect functional impairment following stroke, our study revealed that the automated von Frey test detected substantial and sustained deficit in the withdrawal threshold of the mice on all evaluation days after stroke, whereas the forepaw grip strength test was only marginally sensitive to document functional impairment. Our data demonstrate that the automated von Frey tactile sensitivity test is a time efficient and sensitive test which can be used together with other established tests to evaluate long term functional outcome in the mouse photothrombotic stroke model. In addition, this study should contribute to the ongoing effort to standardize the methods and practices in functional outcome evaluation of mice in different stroke models [4,7].

## 2. Methods

### 2.1. Animals and study design

Twenty, 3 month-old, male CD-1 mice (Charles River Laboratories) maintained in 12-h light/dark cycle and fed ad libitum were used in this study which was approved by the Texas Tech University Health Sciences Center Institutional Animal Care and Use Committee. To minimize handling stress mice were individually handled by two investigators ~2 min once or twice daily for 4–5 days before placing them in plastic compartments designed for the von Frey test (see below) for 15–20 min for habituation. The following day mice were introduced to the forepaw grip strength and von Frey tests for accommodation. Two days after the accommodation trial, baseline assessment of the animals was carried out in all four tests. Following the baseline evaluation, mice were randomly divided into sham and stroke groups (n = 10 per group) and underwent photothrombosis on the third day. Mice were housed individually after stroke, no animal died or was eliminated from the study. Sensorimotor function of the mice was evaluated 1, 7, 14 and 21 days after stroke in the following order of the tests: grid-walking, cylinder, forepaw grip strength and von Frey. The investigators were blinded to the experimental group identity during behavioral assessments and data analysis.

### 2.2. Photothrombotic stroke model

Photothrombosis was induced by illuminating the right hemisphere through the intact skull with cold light (2-mm diameter, 15 min irradiation, fiber optic illuminator light source with a halogen lamp) positioned 1.5 mm lateral from Bregma 0, 5 min after intraperitoneal injection of Rose Bengal solution (8 mg/ml, 10 ml/kg volume) to mice under isoflurane anesthesia at 36.9 ± 0.5 °C [8,21,27]. Sham animals underwent the same procedure except the light illumination. Throughout the text, the left forelimb of both sham and stroke mice is referred to as 'affected forelimb', whereas the right forelimb as 'unaffected forelimb'.

### 2.3. Grid-walking test

Grid-walking test was carried out as previously described [21,27]. Mice walked on an elevated wire grid (12 mm square wire mesh with 33 cm/20 cm total area) for 5 min while being video-recorded. Footfaults for each forelimb and the total normal steps were counted. Footfault index was calculated by: [(#affected forelimb footfaults − #unaffected forelimb footfaults)/(#affected forelimb footfaults + #unaffected forelimb footfaults + #normal steps)].

### 2.4. Cylinder test

Mice were video-recorded in a clear acrylic cylinder (17 cm height and 10 cm diameter) for 5 min to determine forelimb symmetry in exploratory rearing [18,21]. During each rear (at least 20 were required) the use of the affected, unaffected or both forelimbs was counted. Forelimb use was defined as use of the either or both forelimbs to rear and descend from the cylinder wall and the lack of drags during vertical exploratory movements [9]. Forelimb use symmetry index was calculated by: [(#affected forelimb use − #unaffected forelimb use)/(#affected forelimb use + #unaffected forelimb use + #use of both forelimbs)].

### 2.5. Forepaw grip strength test

Grip strength was measured using Mark-10 digital force gauge (model M3-025; Mark-10 Corporation) at peak tension operation mode as described earlier [2,28] with few modifications. After covering one forepaw of the animal with an adhesive tape (~2 × 2 cm piece; M3 Scotch Blue #2090) the mouse was held by the base of the tail above a wire bar (~1 mm diameter, ~3 cm griping surface) connected to the force gauge. The mouse was dangled in a position that allowed it to reflexively grasp the wire with its free forepaw. The investigator then pulled the animal up from the bar at a constant speed and the maximum force generated just before the mouse lost its grasp was recorded. This was repeated five times and each trial was followed by a few second resting of the mouse on the benchtop. The left or right forepaw was chosen at random and its strength was measured in all animals in a row followed by the opposite forepaw.

### 2.6. Automated von frey test

For this test Mark-10 digital force gauge at peak compression operation mode was used, to which a plastic tip (Fisherbrand 02-717-137) was attached. Mice were individually placed in custom made plastic compartments on a metallic mesh (rhomboid shape, 1.3 and 0.7 cm distance between the opposite corners) at ~150 cm elevation and allowed to habituate until exploratory behavior was no longer observed (~15–20 min). The compartments (8 cm W × 17.5 cm L × 6.3 cm H) had three opaque white walls, and a front wall and ceiling made of clear acrylic, allowing accommodation of 5 mice next to each other. The forelimb withdrawal threshold was measured by applying the plastic tip perpendicular to the middle of the plantar surface of the forepaw at constant progressive pressure until the forepaw withdrawal, and the pressure value (g) was recorded [29,30]. This was repeated six times and each trial was followed by a few second resting period. The left or right forepaw was chosen at random and its withdrawal threshold was measured in all animals in a row followed by the opposite forepaw.

### 2.7. Cresyl violet staining and infarct size evaluation

Twenty one days after stroke mice were deeply anesthetized with