Basic Neuroscience

Characterization of long-term functional outcome in a murine model of mild brain ischemia

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HIGHLIGHTS

► We characterized short- and long-term functional outcome in a mouse model of mild brain ischemia.
► Pole test is most suited for evaluation of functional deficits 1–3 weeks after ischemia.
► Corner test and adhesive removal test detect deficits for 3–4 weeks after mild ischemia.
► Catwalk and paw preference test are sensitive long-term tests of clinically relevant deficits.
► Corner rotation is a novel test that improves objectivity and applicability of classic corner test.

ARTICLE INFO

ABSTRACT

Evaluation of functional outcome over the course of several weeks after ischemia is a key component in improving the clinical relevance of experimental stroke studies. Using a battery of behavioral tests, we characterized functional outcome in mice over 4 weeks following 30 min of proximal middle cerebral artery occlusion (MCAo). We evaluated rotarod, chimney, pole and cylinder tests to assess short term functional deficits in a transient stroke model which induces infarcts mainly in the striatum. The corner test, adhesive removal test, cylinder test, catwalk, paw preference test and novel tests of rotation were evaluated for long-term functional outcome. Rotarod detected deficits within the first week and pole test was reliable up to intermediate time points after MCAo. Corner test, adhesive removal test, catwalk and paw preference test detected deficits for up to 4 weeks, as did the novel corner rotation and bowl tests. Chimney and cylinder test did not prove useful in our model of mild stroke. In summary, we established the pole test and rotarod as useful tools to evaluate sensory motor deficits early after mild stroke, and corner test and adhesive removal test at later time-points. Alternatively, corner rotation may be a suitable test of long-term function. Test batteries may be further complemented by catwalk and paw preference test for clinically relevant deficits. There was no correlation of behavioral outcome with lesion size at 28 days, and determining whether these tests are useful for detecting a potential benefit of neuroprotective or regenerative therapies requires further testing.

1. Introduction

With the persisting roadblock in transition from bench to bedside in stroke research, assessment of functional deficits after experimental stroke has become increasingly important (Endres et al., 2008; Fisher et al., 2009; Savitz and Schäbitz, 2008). Just as clinical trials evaluate long-term functional outcome, preclinical stroke research needs to include long-term behavioral outcome evaluation to ascertain whether experimental results bear sufficient clinical relevance. Particularly long-term regenerative and plasticity-dependent treatment approaches require that experimental stroke researchers go beyond mere histological assessment of treatment success (Iadecola and Anrather, 2011;

Murphy and Corbett, 2009). Additionally, while most preclinical studies are conducted in young animals, it is proposed that drug efficacy may be significantly altered in aged animals due to an increased stroke susceptibility and reduced recovery potential (Buchhold et al., 2007; Popa-Wagner et al., 2010), which may further contribute to the lack of successful transfer from bench to bedside.

Concurrently, the use of mice has seen a large surge in experimental stroke research. However, behavioral testing is not well established in mouse stroke models and is far from being standardized (Balkaya and Endres, 2010). While a number of tests have been adapted from the rat, testing methods and principles vary between groups and their results are rarely comparable. Detection of subtle functional deficits several weeks after ischemia has been particularly challenging and needs improvement. Furthermore, it would be advantageous to implement tests that better reflect clinically relevant deficits such as gait and fine-motor ("hand"/paw) function to maximize the potential value of preclinical behavioral testing. As well as establishing new testing methods, it is imperative that the existing tests used in experimental studies be refined and the time windows in which they can be used to evaluate dysfunction and recovery clearly be defined.

The few existing systematic studies of long-term behavioral testing in mouse stroke models examined deficits after more severe ischemia, e.g. 60–90 min transient or permanent MCAo, or distal occlusion (Bouët et al., 2007; Freret et al., 2009; Lubjuhn et al., 2009). We examined the 30-minute filamentous MCAo model in C57Bl/6N mice, which produces a predominantly striatal lesion, selective neuronal cell death and imposes a low animal mortality (Katchanov et al., 2003). As such it may be particularly suited for evaluating long-term regenerative processes (Gertz et al., 2012). But detection of long-term deficits is more challanging due to the fast recovery of animals.

Our study's goals were threefold: (a) to characterize functional long-term outcome in the 30-minute model of transient filamentous middle cerebral artery occlusion, (b) to establish novel and improved testing methods for evaluating long-term outcome, gait and fine-motor function and (c) to contribute toward standardization of methods and practices in functional outcome evaluation in the mouse after brain ischemia. To this end, we selected a number of tests that are widely used in post-stroke testing and assessed the time points at which they yielded significant results in our own model. Moreover, we introduced three novel tests of rotational behavior and applied a classical test of handedness to assess changes in front paw fine-motor function along with an automated gait analysis system to assess subtle gait impairments.

2. Methods

2.1. Animals

All experimental procedures conformed to institutional guidelines and were approved by an official committee (LaGeSo Berlin). Male C57Bl/6N mice (age 8 weeks) were housed in groups of 3–7 mice per cage with a standard light-dark cycle (7 a.m.–7 p.m.) with ad libitum access to food (except as necessary for the paw preference test, see Section 2.4.10) and water. All testing was performed in the late afternoon and evening. Experiments were performed in two cohorts of mice (see Fig. 1). For each cohort, 10–12 animals each were assigned to MCAo or sham operation. One MCAo animal in the first cohort was excluded from analysis as no infarct lesion was found in histology. No postoperative deaths occurred.
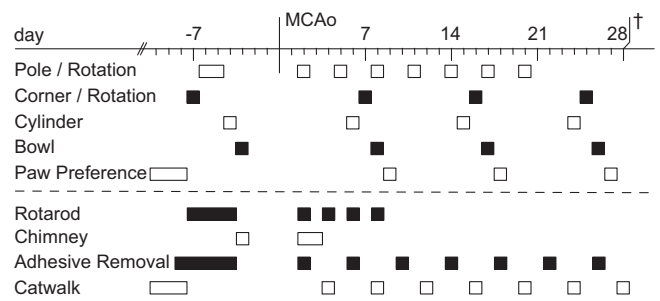

Fig. 1. Experiment design and testing time-points. Tests of the first cohort are listed above the dashed line, tests of cohort two are below. For each cohort 10–12 animals were subjected to MCAo or sham operation. †Euthanasia on day 28 after completion of testing.

2.2. Cerebral ischemia

Mice were anesthetized using 1.0% (vol/vol) isoflurane in 69% N_2O and 30% O_2. Focal cerebral ischemia was induced by 30 min of filamentous occlusion of the left middle cerebral artery (MCA) with reperfusion as described previously (Endres et al., 1998; Engel et al., 2011). For sham operation, the filament was introduced in the left internal carotid artery without advancing it further, and withdrawn after 30 min. Core temperature during the experiment was maintained at 36.5 °C ± 0.5 °C.

2.3. Histologic assessment

After completion of behavioral testing on day 28, animals were deeply anesthetized and decapitated. Brains were removed, snap-frozen, and stored at −70 °C. Lesion size was quantified in 20 μm hematoxylin and eosin (HE) stained cryostat sections by manual delineation of infarcted tissue using MCID Core (Interfocus, Mering, Germany).

2.4. Behavioral testing

2.4.1. Rotarod

Rotarod was performed as previously described (Gertz et al., 2012). In short, animals were placed on an accelerating rotating rod (from 4 to 40 rpm over 300 s) and their latency to fall was recorded. Preoperative training was performed for 4 days with 3 daily trials, the last three trials serving as a preoperative baseline. Postoperative testing was performed every other day for 8 days, 3 trials per day, and the mean was used for statistical analysis. One animal in the control group was excluded as it did not show sufficient motivation to perform the test.

2.4.2. Pole test

The pole test was performed as described by (Matsuura et al., 1997), with minor modifications. The test apparatus consisted of a vertical steel pole covered with tape (Durapore, 3 M) to create a rough surface. Mice were placed head upward on the top of the pole. The time the mouse took to turn completely head downwards ("*t* turn") and the total time it took to descend down and reach the floor with its front paws ("*t* down") were recorded. If the animal was unable to turn completely, the time to reach the floor was also attributed to *t* turn.

Preoperative training was done for 4 days, 4 trials per day. After surgery, animals were tested on the pole test every 3 days starting from day 2 to 20. The average of the 4 trials was used for statistical analysis. Two animals in the control group were not able to perform pole testing correctly and were excluded from analysis.