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Research report

# Assessing gait impairment after permanent middle cerebral artery occlusion in rats using an automated computer-aided control system

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## HIGHLIGHTS

- pMCAO rats were firstly used to assess long-term gait deficits by a computer-assisted method.
- Accurate parameters can illuminate impairment and compensation in rats during walk.
- Average body rotation and propulsion index are first used in cerebral ischemia rats.
- Correlations between the final infarct size and earlier gait deficits were significant.
- Gait analysis is a promising tool to investigate mechanisms of stroke and evaluate potential therapies in rats.

## ARTICLE INFO

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## ABSTRACT

Systematic gait analyses have been widely used in clinical settings as a reliable means of evaluating stroke severity and the efficacy of rehabilitation on people. However, the extent of gait changes post-stroke in experimental quadrupeds remains to be explored. To date, gait studies in cerebral ischemia have been limited to the mild ischemia-reperfusion model. However, studies on pathophysiology and therapy of experimental stroke suggest that permanent middle cerebral artery occlusion (pMCAO) is more similar to naturally occurring cerebral ischemia in humans. This is the first preclinical study to demonstrate that pMCAO rats can be used to assess long-term functional deficits related to gait by a computer-assisted method. Our gait analysis results demonstrate obvious gait deficits in the acute phase of the disease. During recovery, gait function gradually improved, but deficits were still detectable 42 days post-pMCAO. Objective and accurate photogrammetric parameters were used to illuminate laws of impairment and compensation in rats at different stages of cerebral ischemia in injured and uninjured limbs during walking. Compared to previous gait studies involving transient (t) MCAO rats, gait changes observed in pMCAO rats were more similar to changes following naturally occurring cerebral ischemia in humans. Importantly, the average body rotation and propulsion index, not previously used, are specific parameters for accurately assessing gait function during the acute phase of post-pMCAO. Furthermore, the gait test results revealed significant correlations between the final infarction volume and earlier behavioral outcomes. In conclusion, the gait analysis is a promising tool for assessing cerebral ischemia severity, and that it may provide a new means of investigating mechanisms of cerebral ischemia and evaluating potential therapies.



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## 1. Introduction

Ischemic stroke is one of the most frequent causes of death and leading cause of adult disability worldwide [1,2]. Even patients who have survived from stroke, 90% of them suffer permanent neurological deficits. It is essential for stroke survivors to have a lengthy program of rehabilitation, followed by life-long clinical support with rehabilitation therapy in order to improve their life quality [3,4].

However, treatment options are very limited at present and many promising agents used in stroke models have failed to live up to expectations in clinical studies [5]. This may be partly because many preclinical studies have employed a narrow window after the ischemic episode for evaluating functional outcomes in screening therapeutic candidates. These short evaluation periods following injury have significantly limited our understanding due to a lack of information on the delayed effects of treatment, as well as to a short-lived and reversible neuroprotection, generating so-called false positive results. In addition, functional deficits associated with impaired sensorimotor ability after human strokes often develop over the course of months or years [6,7].

Therefore, there is a need to develop a reliable, non-subjective means of measuring outcomes that can detect long-term sensorimotor deficits following middle cerebral artery occlusion (MCAO). Although tests for the locomotion-like rope walk [8], grid walk [9], ladder rung behavior [10] and foot print analysis [11] have unraveled distinct neurological impairments after stroke, their sensitivities are too low to distinguish subtle motor impairments. As we know, gait impairment often occurs as a result of an ischemic stroke. Poststroke gait is characterized by temporal asymmetry, reduced walking velocity, and reduced stride length [12]. Impaired gait function not only reduces ambulation but can also lead to imbalance and falls, especially in elderly patients experiencing a stroke [13]. Analyzing neurological symptoms such as gait impairment may improve evaluation of the neurological outcome, both in humans and animals, and may also aid in the assessment of the effectiveness of new treatment concepts. To date, several groups have investigated gait impairments in different stroke models. In studies of transient ischemia (tMCAO) with rats or mice [14–17], gait parameters were found to be similarly altered, indicating that gait analysis is a feasible method for use with animal stroke models. However, permanent ischemia (pMCAO) without reperfusion is more similar to naturally occurring cerebral ischemia in humans and is thus of greater clinical relevance. One recommendation of the Stroke Therapy Academic Industry Roundtable (STAIR) called for replicating findings in multiple laboratories using pMCAO models, also because pMCAO models could better simulate typical human stroke injury without reperfusion on pathophysiology and therapy of experimental stroke. Moreover, the pMCAO model also provides a useful means for testing therapeutic approaches aimed at repairing the injured tissue in late stages of cerebral ischemia [18,19].

The goal of the present work was to describe gait changes in rats after permanent middle cerebral artery occlusion (pMCAO) and to evaluate results of behavioral tests used to detect long-term gait deficits for up to 42 days after ischemic injury. Several parameters that have not been employed in previous studies were added to observe their usefulness in assessing gait in cerebral ischemia. Furthermore, correlation analyses were carried out between gait parameters and the cerebral infarction volume in order to evaluate associations between gait and cerebral impairment in ischemic stroke, and to identify ischemic models and behavioral tests which could be reliably used for screening in pharmacological studies and in bench to bedside translation.

## 2. Materials and methods

### 2.1. Experimental animals and environmental conditions

Male SD rats (HFK, Beijing, China), 10 weeks old, weighing 300–320 g at the beginning of the experiments, were housed with lights on from 7:00 h to 19:00 h. Surgery was performed after all the rats were allowed to acclimate for 1 week. Rats were housed in groups of four per cage with ad libitum access to food and water. The animals were divided into two groups for use in gait test at all the observing time points. These groups consisted of pMCAO ($n = 8$) and sham ($n = 8$) rats respectively. Room temperature and humidity were maintained at $23 \pm 1\,^{\circ}C$ and $65 \pm 5\%$, respectively.

All experiments were performed in accordance with protocols approved by the Committee for the Care and Use of Laboratory Animals of IMPLAD, CAMS & PUMC, China and were based on the "Principles of Laboratory Animal Care" (NIH publication no. 86-23, revised in 1996) and PR China legislation for the care and use of laboratory animals. Sufficient measures were taken to minimize animal suffering during experiments, such as moving or gripping experimental animals with gentle manipulation, changing litter in cages frequently for animals with adequate food and water, and so on.

### 2.2. Permanent MCAO surgical procedure

Permanent focal cerebral ischemia was induced by occluding the middle cerebral artery using the previously described intraluminal suture method [20] with minor modifications. Briefly, rats were anesthetized with 10% chloral hydrate diluted in physiological saline (3.5 ml/kg, IP). And a monofilament coated with poly-L-lysine (0.1 wt/vol% in deionized water, Sigma, USA), was used to occlude the origin of the middle cerebral artery (MCA). It was inserted into the internal carotid artery lumen until it met mild resistance, approximately 2.2 cm beyond the common carotid artery bifurcation. The suture was secured with a ligature and maintained in place until sacrifice. The same surgical procedure was conducted in sham-operated rats in which the MCA was not occluded. During the surgical procedure, the body temperature was maintained at 37 °C using a homeothermic blanket.

### 2.3. Determination of neurological symptoms

Motor and behavioral changes were assessed one hour after surgery using a five-point scale [21] as follows: 0, no neurological deficit (normal); 1, failure to extend right forepaw (mild); 2, decreased resistance to lateral push (mild to moderate); 3, circling or walking to the right (moderate); and 4, loss of walking or righting reflex (severe).

### 2.4. Gait analysis

Gait assessment was carried out in rats on the 1st, 7th, 21st and 42nd day after pMCAO by the automated computer-assisted method (Xin Hai Hua Yi Instrument Co., Beijing China). Data were collected and analyzed with Gait Analysis Lab software version 5.0. The equipment was located under natural light in a silent room. In brief, the system consists of an elevated 1.2 m-long glass plate which is illuminated with a fluorescent light coming from the side and the fluorescent light is internally reflected in the glass allowing the paws to reflect light as they come into contact with the glass floor. A ceiling on top of the walkway creates a red background to produce the contour of the animal. A high-speed camera (100 frames) underneath the glass plate captures the images which are subsequently analyzed by the connected computer program (Fig. 1A). The video acquisition system is sealed with a PVC sheet to ensure a uniform dark environment to insure controlled lighting in the experiment. Prior to the first testing day, the animals were trained to traverse a glass walkway toward their home cage. On subsequent training days, three complete runs across the walkway were recorded by a camera positioned below (Fig. 1B). If an animal failed to complete a run within 5 s, walked backwards, or reared during the run, the animal was given an additional re-run. Pixels below a light intensity of 20 units on a 0–255 arbitrary scale were filtered out. Prints can be inspected individually and in combinations, and timing diagrams for paw placements are available (Fig. 1C and D). Gait parameters were classified into four main categories as summarized in Table 1.

### 2.5. Histology

The aim of the histological evaluation was to estimate the total infarct volume. After completion of the last behavioral test time-point, rats were sacrificed and transcardially perfused with 4% paraformaldehyde in PBS. Brains were removed from skulls and immersed in paraformaldehyde at 4–8 °C. Each brain was then cut into six coronal 2 mm-thick slices. Histology sections (approximately 5 μm thick) were obtained for evaluation by light microscopy and scanned into the computer. In each section, an outline was traced around the contralateral hemisphere and the ipsilateral hemisphere using Image J (NIH, USA). The infarct area ($mm^2$) was calculated by two independent and experimentally blind assistants by subtracting the area of the ipsilateral hemisphere from the contralateral hemisphere. The infarct volume was calculated as the mean cavity area of two adjacent sections multiplied by the distance between them. The total volume was the sum of the volumes for