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Research report

# The usefulness of operant conditioning procedures to assess long-lasting deficits following transient focal ischemia in mice

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ABSTRACT

In this study, we examined a number of short and long-term sensorimotor, behavioural and cognitive consequences of an experimental ischemia induced by a 60-min right middle cerebral artery occlusion (MCAO) in 129S2 mice. During 14 days after surgery, a classical sensorimotor assessment was conducted using hanging wire test, negative geotaxis test, grip strength test, accelerated rotarod test and locomotor activity-meter. In order to provide a technique for the assessment of more resistant consequences of ischemia on fine psychomotor control, the peak procedure (a modified version of the operant fixed-interval schedule of reinforcement) was used. This procedure also helped to objectify temporal perception in mice five weeks following surgery. On several sensorimotor tests, ischemic mice showed some degree of impairment which rapidly tended to improve after stroke, a profile of results substantially consistent with previous studies. Five weeks post-surgery, ischemic mice tested with the peak procedure exhibited a moderate but yet significant temporal regulation impairment along with a reduced response rate compared to control mice. The present results suggest that the peak procedure and other derived operant schedules of reinforcement may provide useful and sensitive tools for the long-term assessment of both behavioural and cognitive aspects of the consequences of an experimental ischemia.


## 1. Introduction

Acute ischemic stroke constitutes a major cause of mortality and long-term disabilities in humans [24]. According to the damaged area, cerebral vascular accidents may induce sensory, motor, behavioural and cognitive impairments in patients (such as inhibitory control, cognitive flexibility, learning or memory deficits) [31,46,48].

Amongst the available rodent models of experimental ischemia, the middle cerebral artery occlusion (MCAO) is generally considered to be a reliable technique with well known advantages and limitations [10,23]. Several surgical variants exist, which may constitute sources of variability between studies. For example, the occlusion may be carried out on the left or the right artery and even bilaterally. It can be permanent or transient (i.e. the occlusion before reperfusion may be kept from 15 min to 2 h). These variations in surgical parameters can lead to different cerebral and behavioural symptoms or at least, for a given specific function, to various degrees of impairment. For instance, in a study carried out in mice, a right 30-min MCAO has been reported to increase locomotor activity whereas a left one increased anxiety scores (8–10 weeks after occlusion) [56]. By contrast, in another study, neither a right nor a left 90-min MCAO conducted in rats did affect locomotor activity, whereas the left MCAO decreased rearing (until 30 days after the occlusion) and the right occlusion specifically affected learning and memory performance in a radial maze (during approximately 2 weeks after the occlusion) [44]. Obviously, the severity of the impairment depends on the occlusion duration [20,41].

Given that functional deficits in humans may progress months after stroke, several authors have emphasized the importance to evaluate behaviour and cognition at long as well as short delays after stroke in animal research [43]. It is thus now recognized that functional recovering should ideally be assessed at different moments after stroke, not only a few days after the brain vascular accident but also after the process of cell death is completed (at least several weeks after a 30-min MCAO in mouse) [46,56] and even later because functional improvements may require a long time. Parallel histological, behavioural and cognitive measurements in ischemia studies are undoubtedly essential given that it has been reported that functional and histological impairments generally do not correlate [6,9,17,22]. Infarct volume and functional deficit

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may correlate shortly after stroke but this correlation progressively disappears [54].

Unfortunately, most functional tests have been designed to assess stroke-induced sensorimotor deficits within a few days following cerebral ischemia [56]. These were primarily conceived to study the effects of neuroprotective treatments applied right after stroke and mainly assess locomotor activity, postural abnormalities, coordinated movements, balance, forelimb strength or sensory capabilities [31]. Their sensitivity in discriminating ischemic mice from sham controls is often considerably reduced in case of repeated assessment, sometimes early after surgery [23]. Nevertheless, some of these tests, which have not always been used frequently, seem useful to detect subtle impairments several weeks or even months after stroke. For example, the corner test has proved to be one of the most sensitive tests to detect sensorimotor and postural asymmetries in mice 90 days after MCAO [57]. The adhesive-removal test readily reveals sensorimotor deficits several weeks or months after stroke [4,52]. The cylinder test may also constitute an interesting alternative to efficiently assess forelimb use as well as rotation asymmetry [18,26]. However, unpublished results from our laboratory suggest that these tests are not necessarily appropriate for relatively hypoactive mice, such as our intact 129S2 mice, which did not spontaneously produce the expected behaviours.

As regards the assessment of cognitive functions (which may also provide subtle indexes of long-term impairments after ischemia), the results are scarce and conflicting. For example, ischemic (MCAO) mice and rats tested in the water maze task may present significant cognitive deficits lasting several weeks post-surgery [13,14,31,35] or remain indifferent to ischemia, especially when mice are used [4,25,55]. A similar although perhaps better profile of results exists for the passive avoidance task, ischemic rodents presenting a clear-cut deficits in the acquisition or retention of the aversive event in most studies [3,4,17,36] but with some exceptions [15,49]. Note that the sensitivity of the passive avoidance task has been established in mice until four to eight weeks after stroke in rats [2,3]. These discrepancies, whose possible causes have been extensively discussed elsewhere [4], are aggravated by the lack of studies attempting to assess long-term outcomes after focal ischemia, especially in the mouse literature. There is a deep need for tests able to specify the array of functions or sub-functions that have been impaired by a given stroke model and to evaluate how these might recover over time.

The striatum is one of the major neuroanatomical structures impaired by MCAO; depending on the occlusion duration, the infarction may extend to adjacent cortical areas. Because the striatum is implied in fine sequentially-organized motor control, several operant conditioning procedures have previously been used to examine the effects of striatal lesions. For instance, when submitted to an operant differential reinforcement of low rates procedure, ischemic rats (90- or 120-min MCAO) produced longer inter-response times than control rats [20] (this may suggest some temporal perception deficit). Using a response-duration differentiation procedure, it has also been found that fine motor control and timing can both be impaired following unilateral nigrostriatal lesions [21]. Intrastriatal bilateral injections of scopolamine (an amnestic muscarinic antagonist) in rats readily decrease the response rate without affecting the motivation for food or the quality of the temporal perception [1]. Operant conditioning procedures have unquestionably proved valuable in the assessment of the psychomotor and cognitive consequences of striatal lesions.

The present study aimed at assessing the long-term psychomotor and cognitive impairments induced by a transient 60-min right MCAO in the mouse. Along with several classical sensorimotor tests, we elected to use the operant conditioning "peak procedure" which has primarily been conceived for the assessment of time perception. More specifically, this procedure, which is still untapped in the field, is a variant of the classic operant fixed-interval schedule of reinforcement that induces at most of the trials a typical responses distribution characterized by an intra-trial local high rate centred on the reinforced duration. Any motor control impairment should immediately affect the rate of lever presses. Moreover, the temporal distribution of these lever presses may reveal potential impairments of the timing ability. So, the peak procedure may help to detect deficits in the animal's ability to develop high rates of lever presses but also deficits in its ability to build and use temporal representations [7,12].

## 2. Materials and methods

### 2.1. Animals

Two months old male 129S2/SvPas mice (weighting 20–27 g) on the surgery day and born in our colony, were used in the present study. Their parents were purchased from Charles River Laboratories (Brussels, Belgium). In the colony room, lightning was on according to a 12-h light–dark cycle with lights on at 08:00 h and ambient temperatures maintained at 20–22 °C. Mice were group-housed in transparent polycarbonate tubs ($L$ 26 cm × $W$ 40.5 cm × $H$ 20 cm) containing standard pine sawdust bedding. Tap water (bottles) was continuously available in the home cage. Food (standard chow pellets, Carfil, Brussels, Belgium) was also freely available except for the operant conditioning training during which mice were individually housed to allow for the control of the appropriate food diet. The bodyweight of each mouse was then brought at approximately 85 percent of their ad libitum baseline bodyweight using controlled food quantity.

The experimental protocols have been approved by the ethic committee of the University of Liège in accordance with recommendations of the European Community Council for the Ethical Treatment of Animals (EEC Council Directive No. 86/609 of the 24 November 1986).

### 2.2. Surgery

A cerebral infarct was induced by occlusion of the right middle cerebral artery (MCAO). Mice were anesthetized with 1.5–2% isoflurane (BIOSEB, Chaville, France) delivered through a nose mask. Body temperature was monitored using a rectal probe and regulated at 37 °C throughout the surgical procedure with a heating blanket (BIOSEB, Chaville, France). Mice were subjected to cerebral ischemia induced by a 60-min MCAO or to sham surgery (same surgical procedure except for MCA occlusion). The right common carotid artery was exposed through a midline neck incision. Unilateral MCAO was performed by inserting a 6.0 silicone-coated monofilament into the right internal carotid artery [50] until a reduction of the cerebral blood flow was confirmed by a laser Doppler flowmetry probe (Moor Instruments Ltd., Devon, UK) placed on the right parietal bone. Mice showing a regional cerebral blood flow reduction lesser than 80% were not included in the study. Then the neck incision was closed with the monofilament left in place, the anaesthesia was terminated and the mouse placed in a warm recovery cage. After 60-min of MCA occlusion mice were briefly anesthetized with isoflurane and the monofilament was removed. After closing the neck incision, mice were allowed to recover in a warm recovery cage for a couple of hours. Mortality (15%) occurred within 48 h post ischemia. Surviving animals were weighed each day before behavioural testing. At the end of behavioural testing each brain was collected for infarction confirmation via histological analysis. A separate group of 22 mice were sacrificed 24 h after surgery for histological analyses and infarct volume determination.

### 2.3. Infarction analysis

Twenty-four hours after surgery, mice ($n$ = 22) were overdosed with Nembutal. Each brain was carefully removed, frozen in nitrogen vapour and cut in 16 μm-thick coronal sections using a cryostat microtome (LEICA Microsystems, Groot-Bijgaarden, Belgium). Sections (400 μm apart) were stained with 1% hematoxylin/eosin. Infracted area was determined under microscope (NIKON, Analis S.A., Namur, Belgium) using Lucia-G image analysis software (Analis S.A.). Infarct volume was then calculated by multiplying the average of two successive infarct areas by the distance between them [27].

### 2.4. Experimental design

In this study, behavioural tests were conducted from the first to the fifth week post-surgery. Fig. 1 summarizes the planning of each test. Mice were trained at the accelerated rotarod and the grip test 1 day before surgery. All the other tests took place after surgery.

#### 2.4.1. Hanging wire test

This task was used as a measure of grasping ability, forelimb strength and coordination movements [17]. Mice (13 sham and 9 ischemic) used their forelimbs to