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Research report

Assessment of behavioral flexibility after middle cerebral artery occlusion in mice

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HIGHLIGHTS

- Middle cerebral artery occlusion (MCAO) is an animal model of stroke that results in various functional impairments.
- Long-term cognitive deficits induced by MCAO are not well characterized, especially regarding executive functioning.
- We used an operant task assessing behavioral flexibility (a process related to executive functioning) after MCAO in mice.
- Four weeks after MCAO, mice display a deficit in behavioral flexibility.
- Systematic infarction of the dorsomedial striatum could be accountable for the flexibility deficit.

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ABSTRACT

Middle cerebral artery occlusion (MCAO) is the most common animal model of cerebral ischemia and induces various functional impairments. Long-lasting deficits resulting from MCAO however, remain insufficiently characterized, especially regarding cognition. Yet, behavioral flexibility, a prominent cognitive process is found impaired after stroke in humans. We thus used an operant-based task to assess behavioral flexibility in mice after MCAO. Three weeks after 30 min MCAO surgery, mice were subjected to a battery of sensorimotor tests (rotarod, vertical pole test, spontaneous locomotion and grip-strength test). Behavioral flexibility was then assessed in an operant task, in which mice, rewarded according to a FR5 schedule of reinforcement, had to alternate their operant responses between two levers from trial to trial. Regarding sensory and motor functioning, only the pole test yielded a significant difference between MCAO and sham mice. In the operant flexibility task, results showed a behavioral flexibility deficit in MCAO mice; neither the operant response acquisition nor the appeal for food rewards was altered. In conclusion, our operant-based task revealed a long-lasting behavioral flexibility deficit after MCAO in mice.

1. Introduction

Cerebral stroke is one of the leading cause of mortality in industrialized countries and the first cause of adult disabilities [1]. This led to the development of various animal models which reproduce stroke etiologies and consequences (see [2] for a review); most of them inducing an ischemic episode, as cerebral ischemia accounts for 85% of stroke [3]. Unfortunately, despite the multiplicity of stroke models, none of them managed to correctly predict the efficacy of putative neuroprotective compounds in clinical trials. Recombinant tissue plasminogen activator (rt-PA) is the only clinically proven pharmacological stroke treatment, but it can only be used for blood clot thrombolysis within a limited time-frame and only apply to very specific stroke models [4]. Stroke research has since been characterized by a "translational roadblock", as it seems unable to cross the bench-to-bedside frontier when it comes to neuroprotection [5].

Several flaws in the design of experimental stroke studies have been pointed out to explain this lack of positive results: non-blinded functional or histological assessment [6], non-randomized allocation to experimental groups [7], test populations generally restricted to young and healthy animals (while in human stroke strikes rather elderly or other risky populations) [6,8], uncontrolled physiological variables (such as blood gas pressure or body

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temperature) [9], inappropriate stroke model regarding the therapeutic target [10] or investigation limited to rodent models only, seldom completed by studies on non-human primate [11].

Series of recommendations and guidelines have recently been established to improve practical and translational aspects of stroke research [9,12–15]. Among them, one major criticism justifying the failure of translational neuroprotection studies is often brought forward: the lack of long-term functional outcome assessment. Ischemic brain infarctions evolve over time and it is generally admitted that the final infarct size can only be determined around 21 days after ischemia [16]; furthermore, some authors suggested that neuroprotective compounds could have delayed effect in terms of neuronal death reduction [8,17,18]. For these reasons, systematic inclusion of long-term measurements is strongly suggested [5]. Additionally, if functional outcome after stroke is not carefully taken into account, preclinical research might lead to the development of neuroprotective therapies that improve histopathological aspects (e.g., reducing the infarct size) without ameliorating the functional status, whereas the later should be the endpoint of any therapeutic treatment. Hence, outcome analyses should present the results of a functional assessment including sensory, motor and cognitive measures [5,9,17]. But while stroke experts acknowledge the paramount importance of behavioral evaluation, they face a lack of agreement concerning the tests required to appropriately assess sensorimotor and cognitive abilities [19,20].

Middle cerebral artery occlusion (MCAO) is the most widely used model of ischemic stroke for different reasons: it induces a focal lesion, allows reperfusion and, more importantly, affects the middle cerebral artery (MCA), the most frequent origin of stroke in humans [3]. Transient MCAO leads to mostly subcortical (i.e., basal ganglia) infarcts that can extend to other cerebral structures, depending on occlusion duration.

Regarding functional outcome of MCAO, behavioral evaluation is often restricted to sensory and motor abilities. Commonly used tests – such as rotarod [21–23], open-field exploration [24,25] or neurological scales [26–28] – were successful in identifying significant impairments after MCAO, and even proved to be sensitive to the effect of some neuroprotective agents [29,30]. However, they are sometimes criticized for their lack of sensitivity due to spontaneous recovery or compensatory strategies [31–35]. Therefore, they might not be suited for the identification of permanent disabilities, such as those encountered in human stroke victims. It is nevertheless interesting to plan those tests before the long-term assessment of other behavioral or cognitive feature, because they can contribute to objectify some specific recovering at different levels (general functioning, locomotor and exploratory activity, etc.). Other sensorimotor tasks assessing finer and more subtle stroke-related deteriorations seem to be more reliable and suited for a long-term use: the sticky tape test [21,36], the corner test [33,37], the staircase test [32,38], composite neurological scales [39] or other fine motor skill assessments (e.g., digit rating scale) [35,40]. For instance, the staircase test is able to discriminate MCAO from sham mice up to 9 weeks [32]. In counterpart to their long-lasting sensitivity, these tests are effortful in terms of testing and scoring.

Beside sensory and motor abilities, cognition is less systematically investigated in experimental stroke studies, despite that long-lasting cognitive impairments are often reported after stroke in humans [41–43], and that MCAO can affect components of the limbic system (such as the hippocampus). The active/passive avoidance paradigm and the Morris water maze (MWM) are the most common tests used to assess cognition after MCAO [4,44], both appraising associative learning and memory processes. In rodents, passive avoidance revealed an impairment in the avoidance response acquisition and retention, at both short and delayed time-points after MCAO [31,34,45,46]; it also seems to be sensitive to pharmacological therapy [47,48]. However, passive avoidance also occasionally failed to reveal any stroke-induced cognitive deficits [23]. The consequences of MCAO on water maze performance show some inconsistencies depending on which specie is used as a model. If rat navigational skills seem to be consistently affected by MCAO [49–51], the consequences are more controversial in mice: while some studies report a deficit for platform location learning [52,53], other did not find any difference between MCAO and sham mice for neither learning or short term retention [31,54,55].

However, it has been pointed out that the kind of tasks usually used in combination with rodent models (such as the MWM) do not address any of the most usually altered functions in patients [56]. Indeed, cognitive outcome after stroke are much more diversified. Language disorders are common, but tend to spontaneously recover [57,58], while attention-related deficits (especially affecting the contralateral side) are more persistent [57,59]. Memory impairments constitute a more debated issue: if alteration in procedural (or implicit) memory seems to be a frequent symptom amongst MCA stroke patients [60,61], declarative (or explicit) memory processes can be shown to be altered in some cases [62], but considered as spared in others [63–65].

A handful of studies proved that operant conditioning permits to underline various persistent behavioral and cognitive deficits in animal after cerebral damages. Craft and colleagues [66] demonstrated that performance in an operant task assessing sustained attentional performance was impaired following multiple microsphere-induced embolisms. In a lateralized reaction time task, Hoff et al. [67] identified a form of contralateral neglect, similar to attentional disorders encountered in stroke patients. In an operant-based peak procedure, Ferrara et al. [68] showed that right MCAO in mice significantly reduced bursts of lever-pressing around the estimated time to reinforcement; a result in line with other independent studies indicating a fall in responses rate after focal or global ischemia in rats [69,70].

Impairments in executive functioning, a set of high-order mental processes, are also common after ischemic stroke [71,72], and more importantly, are good predictor of long-term disabilities [42]. In particular, behavioral (or cognitive) flexibility is one of the most prominent executive functions and allows us to adapt our behavior and cognitive sets to changing situations. More specifically and importantly for the translational aspect of the animal model of stroke, flexibility deficits have been repeatedly identified in humans after vascular lesions in the MCA territory [72], whether using paper-and-pencil neuropsychological tests (such as the Trail-making test B [62,73], verbal fluencies [62] or the Wisconsin Card-Sorting test [72,74]) or in more complex computer-assisted flexibility tasks [75]. Surprisingly, behavioral flexibility deficits have not been extensively investigated after MCAO while operant conditioning derived procedures have been advantageously used to assess that specific cognitive function in rodents. Combining animal stroke models and operant conditioning techniques to assess cognition yet seems feasible and offers numerous advantages (automaticity, objective measurements, parallel to the human neuropsychological evaluation, etc.), but one must be careful to ascertain beforehand that animals do not display major sensorimotor impairments that would prevent them to properly perform the task.

In the present study, along with other classical tests appraising sensorimotor status (rotarod, vertical pole test, grip-strength test and spontaneous locomotion), we used a simple operant-based task to assess behavioral flexibility. MCAO and sham mice were subjected to a task requiring them to constantly adapt their behavior throughout the session. Namely, mice had to switch between two alternatively active levers in order to obtain food rewards. We hypothesized that animals would display impaired behavioral flexibility after MCAO.