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Research report

Evaluation of neurobehavioral deficits following different severities of cerebral ischemia in rats: A comparison between the modified hole board test and the Morris water maze test

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HIGHLIGHTS

- Histologic outcome after various cerebral ischemia models in rats is well described.
- Clear evidence on the effects on executive functioning is still missing.
- This study compares the MWM with the modified hole board test (mHB).
- The mHB accurately reflects different severities of histological damage.
- The mHB is more sensitive for evaluation of cognitive–behavioral performance.

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ABSTRACT

Objective: While histological injury following cerebral ischemia has been extensively characterized in rodents, evidence on the effects on executive functioning is still missing. This study was designed to evaluate neuropsychological outcome following different severities of cerebral ischemia in rats with the modified hole board test or the Morris water maze.
Setting: With institutional review board approval, anesthetized rats were exposed to bilateral carotid artery occlusion (BCAO) for escalating time intervals (0–12.5 min). Postoperatively cognitive performance was assessed using either the modified hole board test (mHB) or the Morris water maze (MWM). On postoperative day 14 rats were euthanized and intact neurons in five cerebral regions were counted.
Results: Rats of the 0 and 5 min groups showed normal functional outcome with mild histological damage after 5 min of BCAO. Following 7.5 min of BCAO the mHB test showed cognitive deficits reflecting histological damage of the hippocampus while the MWM revealed normal functional outcome. Rats of the 10 and 12.5 min groups showed cognitive deficits in both tests, motor dysfunction and behavioral alterations in the mHB test and profound histological damage.
Conclusions: The results indicate that the mHB is not inferior to the MWM for the evaluation of cognitive impairment following incomplete forebrain ischemia in rats. As the mHB additionally investigates a variety of behavioral dimensions and motor parameters in the same test environment, it is advantageous for the evaluation of interacting and potentially confounding behavioral changes following cerebral ischemia in rats.

1. Introduction

Neurologic and cognitive impairments following cerebral ischemia during anesthesia and surgery remain as common and severe complications [1–5]. Therefore, a number of animal models of cerebral ischemia have been developed to investigate the underlying mechanisms. With respect to histopathology, these models have been thoroughly characterized, showing that the hippocampus, especially the CA1 region, is a key structure for learning and memory, showing a high vulnerability to cerebral ischemia [6]. While mechanistic studies have focused on bio-molecular alterations during a short postischemic interval, studies investigating neuroprotection as primary endpoint require a long term assessment of neurocognitive performance [7]. Therefore, to investigate

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Table 1
Cognitive, behavioral and motor parameters assessed with the two tests.

Hole board testing				
	Memory system		Putative mediating brain area	Hole board behavior
Cognitive processes	Visuo-spatial long-term memory		Hippoampus	Visit of a non-baited hole (=wrong choice) [number per trial] Non-visit of a baited hole (=omission error) [number per trial]
	Visuo-spatial short-term memory		Prefrontal cortex	Revisit of a baited hole (=repeated choice) [number per trial]
	Cognitive performance (motivation)			Time needed to perform the trial (=time trial) [s]
Behavioral Processes	Anxiety-related			% Time spent on the board (=time on board) [% of time trial] Latency to 1st board visit (=latency board) [s] Number of board entries (=board entries) [number per trial] % Time spent immobile (=immobility) [% of time trial]
	Social affinity			% time spent in group contact [% of time trial]
	Exploratory motivation	• Directed	• General	Latency to 1st hole visit (=latency hole visit) [s] Number of holes visited (=hole visits) [number per trial] Rearings [number per trial]
	Physiological arousal			Defecation and urine (=boluses) [number per trial] % of time spent grooming [% of time trial]
Motor Function	Locomotor activity Fine motor parameters			Line crossings [number per minute trial] Motor dexterity (=unsuccessful hole visits) [number] Motor skill (=time food intake) [s]

Morris Water Maze		
Cognitive processes	Visuo-spatial long-term memory	Time to find the platform (=latency platform) [s] Distance to find the platform (=distance platform) [m]
Behavioral processes	Anxiety-related	Time spent in the marginal area (=thigmotaxis) [% of time trial]
Motor function	Locomotor activity	Swimming speed [m/s] Line crossings [number per minute trial]

the contributing factors and to screen potential neuroprotective strategies in a preclinical setting, a cognitive–behavioral test suitable for the detection of longer lasting neurocognitive deficits is required.

Several neurobehavioral tests are available for rodents, with the Morris water maze (MWM) as a commonly used test for assessing memory deficits following hippocampal injury [6]. Although being a well established and widely accepted learning task it is controversially discussed [8]. It is adapted to the study of rapid allocentric spatial learning, but less suitable for repeated measures or for assessment of long-term memory deficits. The modified hole board (mHB) test has been shown to be a differential and non-stressful cognitive–behavioral test in mice and rats [9–15]. Even though it has already been used to assess neurocognitive outcome after cerebral ischemia in rats [16–18], it has not yet been compared to an established test paradigm. Therefore, the current study was designed to compare the MWM and the mHB test regarding the ability to assess neurocognitive outcome after various severities of incomplete forebrain ischemia in rats.

2. Material and methods

2.1. Study design

Experimental protocols were approved by the institutional animal care committee (Regierung von Oberbayern, 80534 München, Ethical Committee no.: 209.1/211-2531-107/04, Dr. B. Wirrer, on March 22, 2005) and all procedures described herein met the guidelines of the National Institutes of Health for animal care.

Rats were housed under standard laboratory conditions (12 h light/12 h dark, lights on at 0:30 a.m., 22 °C, 60% humidity and free access to water and standard rat chow) three weeks prior to the experiments for acclimatization to the changed day–night-rhythm. Ten days prior to surgical preparation 148 animals were randomly assigned to either the mHB test group or the MWM group. Animals from the mHB group were housed in home cage compartments (size: 80 cm × 60 cm × 50 cm) next to the mHB test environment, animals from the MWM group were housed in standard macrolon cages (size: 55 cm × 33 cm × 20 cm). All animals were housed in social groups of 6 individuals for the mHB test and 3 individuals for the MWM. The two experimental groups were further subdivided into six subgroups exposed to escalating durations of incomplete forebrain ischemia (0, 5, 7.5, 10 or 12.5 min) or served as untreated controls ($n = 10$ in each subgroup).

As main endpoints motor and cognitive performance over 14 postoperative days were defined. Consequently, rats not surviving the observation period or rats that had to be euthanized (due to poor general condition, weight loss over 20%, persistent postischemic seizures), had to be excluded due to missing data and were replaced to keep the sample size equal ($n = 10$).

2.2. Surgical preparation and incomplete forebrain ischemia

Non-fasted male Sprague-Dawley rats from Charles River Laboratories (325–400 g, 10 weeks old; Sulzfeld/Germany) underwent surgery for incomplete forebrain ischemia with reperfusion as previously reported [19,20]. Briefly, the tail artery was cannulated for blood pressure monitoring and the right external jugular vein for blood withdrawal. The common carotid arteries were exposed and encircled with suture, leaving the vagal nerves and cervical sympathetic plexus intact. During surgery anesthesia was maintained with 1.5–2% isoflurane and repetitive fentanyl boluses (5 μg). Cortical electric activity was monitored using subdermal electrodes placed over the parietal and occipital cortex bilaterally and one ground lead placed in the left groin. Pericranial temperature was monitored and servoregulated at 37.5 °C. After surgical preparation and heparinization (50 IU), 0.2 mg cisatracurium was given to provide muscle paralysis during ischemia. Ischemia was induced by withdrawal of 6–8 ml blood via the jugular vein catheter in a heparinized (50 IU) syringe. After mean arterial pressure (MAP) was reduced to 27–30 mm Hg, both carotid arteries were occluded with aneurysm clips. Ischemia persisted for 5, 7.5, 10 or 12.5 min and was confirmed by the presence of an isoelectric EEG. MAP was maintained at 27–30 mm Hg by withdrawal or reinfusion of blood as necessary. To discontinue ischemia, the aneurysm clips were removed and shed blood was reinfused. At this time, ventilation was adjusted to normalize arterial blood gases. The 0 min animals were exposed to the same surgical and anesthetic protocol, but were neither exposed to hypotension, nor to bilateral carotid artery occlusion (BCAO), and served as sham-group. Following reperfusion, rats remained anesthetized with 2% isoflurane, intubated and ventilated for 1 h. After resuming spontaneous