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Research report

A sensitive and reliable test instrument to assess swimming in rats with spinal cord injury

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HIGHLIGHTS

- Eight parameters of swimming in rats with spinal cord injury were evaluated for 8 weeks.
- Six parameters were chosen for the Karolinska Swim Assessment Tool (KSAT).
- KSAT correlated to injury severity and spared tissue after mild to severe injuries.
- KSAT was highly reliable with high intra- and interobserver reproducibility.
- KSAT is a useful instrument for functional studies of spinal cord injuries in rats.

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ABSTRACT

For clinical translation of experimental spinal cord injury (SCI) research, evaluation of animal SCI models should include several sensorimotor functions. Validated and reliable assessment tools should be applicable to a wide range of injury severity. The BBB scale is the most widely used test instrument, but similar to most others it is used to assess open field ambulation. We have developed an assessment tool for swimming in rats with SCI, with high discriminative power and sensitivity to functional recovery after mild and severe injuries, without need for advanced test equipment. We studied various parameters of swimming in four groups of rats with thoracic SCI of different severity and a control group, for 8 weeks after surgery. Six parameters were combined in a multiple item scale, the Karolinska Institutet Swim Assessment Tool (KSAT). KSAT scores for all SCI groups showed consistent functional improvement after injury, and significant differences between the five experimental groups. The internal consistency, the inter-rater and the test–retest reliability were very high. The KSAT score was highly correlated to the cross-section area of white matter spared at the injury epicenter. Importantly, even after 8 weeks of recovery the KSAT score reliably discriminated normal animals from those inflicted by the mildest injury, and also displayed the recovery of the most severely injured rats. We conclude that this swim scale is an efficient and reliable tool to assess motor activity during swimming, and an important addition to the methods available for evaluating rat models of SCI.

1. Introduction

Spinal cord injury (SCI) impairs motor, sensory and autonomic functions, with severe social, economical and health-related problems for the injured person. Whatever treatment strategy that is studied, functional improvement is the outcome relevant for a clinical treatment. Since there are no alternatives to animal studies as the main scientific preclinical basis for potential new treatments, it is critical that these animal studies are evaluated using appropriate and validated functional tests. Since it is inherently difficult to determine what functions in animal models are most relevant to the human SCI patient, the experimental studies should include assessment of different functions.

The importance of investigating several functions in preclinical rodent studies has also been stressed by many investigators [1,2]. Due to technical difficulties, sensory and autonomic functions in rodents are seldom studied although they may be equally important as motor function research. Spontaneous recovery of locomotion, mainly mediated by plasticity in the damaged nervous system [5–7] and remodeling of spared spinal tracts [8], may

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give rise to different outcomes in different species. Therapeutic interventions can lead to recovery of different components of locomotion, since the plasticity evoked will have unpredictable effects on specific spinal tracts and intraspinal circuitry mediating different functions. Nevertheless, functional assessment of rodent model of SCI often focuses only on motor functions of ambulation [3,4].

Many tests of rodent motor functions are also restricted to animals with mild to moderate SCI, while clinical trials most likely will involve patients with severe injuries [13]. Whether a treatment effect observed after mild SCI in rodents can be extrapolated to severe injuries has not been systematically studied, and will inevitably depend on the mechanism of the treatment. Spontaneous recovery after SCI is pronounced in rodents. Rats with moderate injuries show near-complete paralysis the first week after injury but often re-gain weight-bearing capacity during the following 2–3 weeks. Consequently, methods for functional assessment in long-term studies should be applicable to a wide range of impairment. Sensitive and reliable methods for analysis of motor functions in animals with mild, moderate and severe SCI are therefore needed.

For assessment of ambulation after low thoracic SCI in rats – the most common SCI model – the BBB scale [14] has become a standard tool. It is also one of the few tests thoroughly validated for rat SCI. The BBB scale is often combined with other locomotion tests such as gait analysis, grid walk, beam walk, etc. However, translational SCI research would benefit from standardized testing strategies that include tests to assess other functions. Swimming is a motor activity different from walking/open field ambulation. In contrast to walking and running, which are quadrupedal motor activities, normal swimming in rodents is a bipedal movement performed at higher frequency with a strict left-right alternation. Thus, swimming represents a suitable motor activity to be assessed after SCI, in order to broaden the test of motor functions.

Analysis of swimming after SCI has been used in a few previous studies. In a study by Liebscher and colleagues [16] swim performance in rats with SCI was assessed by a combination of parameters. However, the authors presented no evaluation of the method. More recently Zörner et al. [17] published a detailed analysis of motor activity during walking, wading and swimming in SCI rats, but the study did not suggest and validate any specific tools or assessment methods for analysis of SCI. To the best of our knowledge, the Louisville Swim Scale (LSS) is the only validated method for systematic quantitative assessment of swim performance in SCI animals [18]. The parameters used in LSS represent key components of swim performance. The LSS is most accurate for moderate injuries, while the sensitivity to changes after mild or severe SCI is more limited. For example, LSS does not consistently discriminate between mild SCI and non-injured control rats [18]. The aim of our study was to evaluate a number of possible parameters of swim performance, analyze how they are best assessed and combined, to develop a reliable and validated assessment tool for swimming performance, for rats with mild, moderate and severe SCI.

2. Materials and methods

2.1. Animals and surgical procedure

Thirty-six three-months-old female Sprague-Dawley rats (180–220 g, Taconic, Denmark) were housed in a standardized environment with water and food ad libitum, room temperature maintained at 22 °C, humidity at 45–55% and a 12/12 light–dark cycle with lights on at 07:00. Three rats were kept in each cage. All experimental procedures followed the guidelines of the Swedish animal protection legislation, and were approved by Southern Stockholm animal ethical board.

Atropin (0.05 mg/kg, NM Pharma AB) was given 30 min before surgery. Rats were anesthetized with a mixture of Hypnorm (fentanyl citrate, 0.22 mg/kg, and fluanisome, 6.8 mg/kg, Janssen Pharmaceuticals) and Dormicum (midazolam, 3.4 mg/kg, Hoffman-La Roche). Body temperature was kept at 37 °C throughout the surgical procedure. Spinal cords were surgically exposed by a laminectomy at Th9 after placing a few drops of Xylocain (lidocain hydrochloride 20 mg/ml, AstraZeneca) on the vertebra. The exposed spinal cord was subjected to clip compression during 30 s of either of four different forces for (20 g, 45 g, 75 g, 140 g; $n = 7$, 9, 7, 9). One layer of Lyoplant (B/Braun Aesculap AG) was placed on the spinal cord before the wound was sutured. Rats were subcutaneously injected twice with 3 ml Ringer/2.5% glucose (Sigma) before and after surgery. After surgery, the rats were given Temgesic (buphrenorphin, i.m. 7 μg/kg, Reckitt & Colman) as analgesia twice a day for 4 days. The urinary bladders were emptied manually 2–3 times per day, and Borgal (trimetoprim sulfa, s.c. 15 mg/kg, Intervet International B.V.) was administered if signs of urinary infections were seen. Two rats from the 140 g clip compression group were sacrificed due to weight loss larger than the termination criteria set by Karolinska Institutet veterinary guidelines. Four uninjured animals served as controls.

2.2. Testing apparatus

A 150 cm long, 15 cm wide and 30 cm deep Plexiglas swim tank was used (see Suppl. Fig. S1). A partially submerged platform was placed at the end of the tank, allowing even severely injured rats to get out of the water on their own after each session. The tank was filled with tap water (28–32 °C) to a depth of 20 cm. For evaluation of the swimming rat from below, a mirror (100 cm × 14 cm) was positioned below the tank at an angle of 45°. A HD video camera in front of the tank set to a frame rate of 120 s^{-1} recorded swimming in the middle 60 cm part of the tank. Motion sequences and individual frames were evaluated using a QuickTime player.

Supplementary Fig. S1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbr.2015.05.004

2.3. Pretraining and functional testing

Before testing was begun, all rats were accustomed to the test situation by swimming five runs per day for 1 week. At the end of the week they showed no signs of stress during swimming. Rats are natural swimmers using a strict left-right hindlimb alternation with forelimbs held still under the chin. The last day of training all rats but one (excluded) showed this normal swim performance. The rats were also exposed to the open field stage, a 100 cm × 90 cm elevated flat surface, one day before surgery. A BBB score was determined using the protocol of Basso et al. [14]. All rats received the maximal BBB score of 21.

During the test period, the assessment of open field ambulation was done in the morning while swim performance was studied in the afternoon, once a week for 8 weeks after surgery. Upon completing a run of swimming, rats were immediately taken out of the pool and allowed a 20 s rest. Evaluation and scoring of the swim parameters were performed by blinded evaluators watching videos, to avoid that other behavioral signs and symptoms affected the evaluators' expectances of the swim performance. The evaluators engaged in the assessment of inter-rater reliability received no advance training in performing the KSAT, only written instructions, scoring sheets and coded videos.

2.4. Swim performance parameters

We evaluated movements during swimming, and the possible parameters to assess these movements. In previous studies on