Chapter 21

Neurological Exam in Rats Following Stroke and Traumatic Brain Injury

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Abstract

Using the appropriate model for testing neurological symptoms in rats is essential for the assessment of functional outcome. A number of tests have been developed to quantify the severity of neurological deficits. These tests should meet criteria such as validity, specificity, sensitivity, and utility. Although analysis of motor function shows homology in primates and rodents, the total neurological exam scores may not always reflect the clinical outcome. Therefore, the selection of the appropriate tests has critical importance when evaluating therapeutic strategies. This chapter describes Toklu's modified neurological exam score method which can be used practically to assess neurological symptoms following traumatic brain injury (TBI) and stroke. The method is a combination of balance, muscle strength, coordination, and reflex.

Key words Neurological exam, Neurological score, Rat, Brain injury, Stroke, Trauma

1 Introduction

1.1 Assessing Neurological Deficits

A neurological exam typically provides information on the structural and functional integrity of the nervous system. Even though neurological examinations in animals are less sophisticated than those performed on humans, these examinations are still useful to assess the overall performance of humans following conditions such as traumatic brain injury (TBI) and stroke [1]. Using an appropriate model for testing neurological symptoms in rats is essential for the assessment of functional outcome. A number of tests have been developed to quantify the severity of neurological deficits. These tests should meet criteria such as validity, specificity, sensitivity, and utility. Although analysis of motor function shows homology in primates and rodents, the total neurological exam scores may not always reflect the clinical outcome. Therefore, selection of the appropriate test has critical importance when evaluating therapeutic strategies [2–4]. This chapter describes Toklu's modified neurological exam score method which can be used practically to assess neurological symptoms following traumatic brain injury and stroke.

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2 Materials

2.1 Animals

1. This neurological exam scale was tested on different strains of rats (Sprague Dawley, Wistar albino) of both sexes, 2–3 months of age, weighing between 250 and 350 g.
2. Upon arrival in the animal facility, animals were acclimated for at least 1 week. Animals were maintained on a 12:12-h light-dark cycle and provided standard rat chow and water ad libitum throughout the experimental protocol.

2.2 Drugs Used for Anesthesia for the Induction of Neurotrauma

1. Ketamine HCL (Ketalar®, Pfizer, Turkey) 100 mg/ kg ip.
2. Chlorpromazine (Largactil®, Eczacibasi, Turkey) 1 mg/ kg ip.
3. Isoflurane USP (Piramal Critical Care, Inc., Bethlehem, PA) 3%.

3 Methods

3.1 Toklu's Modified Neurological Exam in Rats

Since functional scoring is important in testing neuroprotective drugs, a simple set of criteria common for neurological tests was used to assess the normal and abnormal function in rats that have neurotrauma. Toklu's modified neurological exam is a combination of Bederson's neurological function test [5] and Garcia's behavior score [6]. This test was first used to access neurological scores of rats with encephalopathy due to sepsis [7]. Later, we used this test in brain injury models such as ischemic stroke, weight drop trauma, and overpressure blast injury [8–12].

3.2 Experimental Procedure

1. A 20-point score was used to assess motor and behavioral deficits (Table 1). The higher the score, the more severe the neurological deficit. The sequence of testing animals for a given task was randomized for the animals. All observations were performed by an experienced blind investigator (*see* **Note 1**). In summary, the consciousness, performance in a smooth surface climbing platform (45°), extremity tonus, walking and postural reflexes, circling, and response to the nociceptive stimuli were assessed.
2. For walking and posture, the rats were observed as they moved around freely in their cages (*see* **Note 2**).
3. Finally, the responses to the nociceptive stimuli were assessed by pinching the tail.

Table 1
Toklu's modified neurological exam

Consciousness	Normal: 0 Agitation: 1 Lethargy: 2 Stupor: 3 Coma: 4
Walking/posture	Normal: 0 Limb adduction: 1 Hypomobility: 2 Turning to the paretic side: 3 Spontaneous circling: 4 No postural reflex: 5
Climbing platform	Climbing: 0 Staying on the hind limbs: 1 Hanging for >5 s: 2 Hanging for <5 s: 3 No catching reflex: 4
Rotation test (circling behavior)	>1 s 180°: 0 <1 s 180°: 1 1–3 s 90°: 2 <1 s 90°: 3 1–3 s 45°: 4 <1 s 45°: 5
Response to the nociceptive stimulus	Normal response to the nociceptive stimulus: 0 Hypoactive to the nociceptive stimulus: 1
Total score	Max. 20

3.2.1 Climbing on the Inclined Plane

1. The inclined plane evaluates the ability of the animal to maintain its body position, i.e., balance and equilibrium.
2. The dimensions of the wooden platform are 75 cm length × 15 cm width × 1 cm height as shown in Fig. 1. The platform is placed with an angle of 45° leaning toward the wall.
3. Rats are then placed in the middle of the inclined platform and assessed for their ability to maintain their balance and observed for their climbing behavior.
4. In the inclined platform test, normal rats easily climb up the platform, while severely injured rats slide down the platform.

3.2.2 Circling/Rotation Test

1. This test is used to detect equilibrium deficits and hemiplegia.
2. A custom-made drawing was used for the measurement of the angle in the rotation test. The drawing shown in Fig. 2 is printed on A4 size paper.

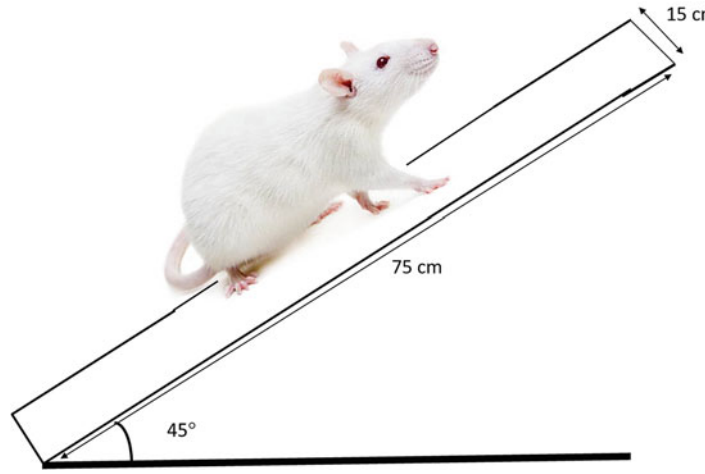

Fig. 1 Wooden platform used for climbing on the inclined platform. The dimensions of the wooden platform are 75 cm length × 15 cm width × 1 cm height. Rats are placed in the middle of the inclined platform and assessed for their ability to maintain their balance and observed for their climbing behavior. In the inclined plane test, normal rats easily climb up the platform, while severely injured rats slide down the platform

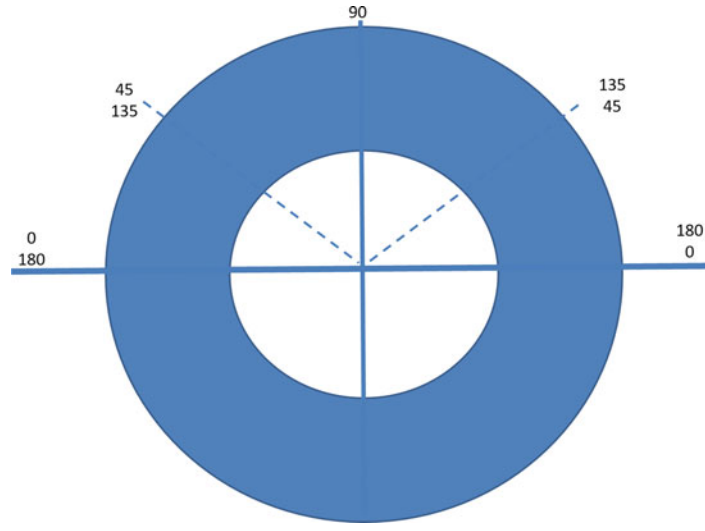

Fig. 2 Measurement of the angle of rotation for circling test: a custom-made drawing was used for the measurement of the angle in the rotation test. The rats were held gently by the tail, suspended 1 m above the floor, and observed for forelimb flexion. Normal rats are expected to extend both forelimbs toward the ground. The degree of rotation and the reaction time were also measured to detect equilibrium deficits and hemiplegia

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3.3 Stroke and Traumatic Brain Injury Models Used with Toklu's Modified Neurological Exam Test

Ischemic stroke and traumatic brain injuries are the main causes of long-term neurological deficits and disabilities. Depending on the location and severity of the lesion, both insults can lead to impairment of sensory, motor, and cognitive function, as well as death (*see* **Notes 3** and **4**).

We used three distinct models to induce neurotrauma: subarachnoid hemorrhage model, weight drop model, and overpressure blast model.

3.3.1 Induction of Ischemic Stroke with Subarachnoid Hemorrhage (SAH) Model

1. Ischemic stroke in rats can be induced by (1) ligation/occlusion of cerebral arteries (the most common one is the middle cerebral artery (MCA) occlusion), (2) induction of photothrombosis, (3) embolic stroke models (microsphere-/macrosphere-induced stroke models and thromboembolic clot models), and (4) injection of endothelin 1 [13, 14].
2. To test the efficacy of the neuroprotective agents, we used thromboembolic clot model induced by autologous blood injection (Fig. 3) [9, 10]. To summarize, in the anesthetized (ketamine 100 mg/kg and chlorpromazine 1 mg/kg IP) rats, a small incision was made in the area of the occipitocervical junction to expose the atlanto-occipital membrane. After the cisterna magna was tapped with a 27-G needle, 0.3 mL of cerebrospinal fluid was gently aspirated. Freshly drawn blood (0.3 mL) collected from the femoral artery was then injected into the cisterna magna within a 2-min period. Immediately after the injection of blood, the puncture site was sealed with tissue glue to prevent the formation of a fistula. To permit blood distribution around the arteries, each rat was tilted at an angle of 20° for 30 min with its head lowered.

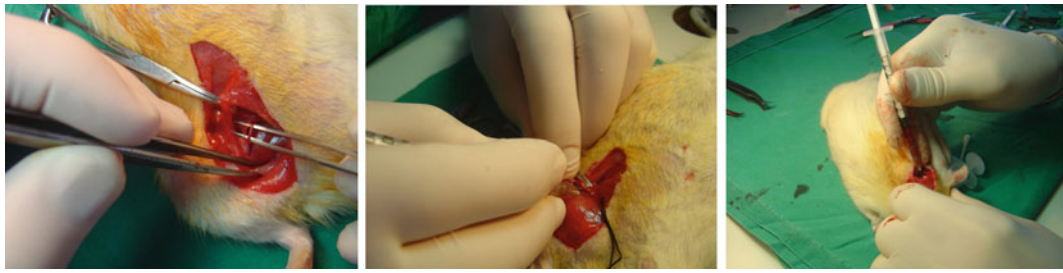

Fig. 3 Subarachnoid hemorrhage model: the cisterna magna was tapped with a 27-G needle, and 0.3 mL cerebrospinal fluid was gently aspirated. Freshly drawn blood (0.3 mL) collected from femoral artery was then injected into the cisterna magna within a 2-min period

3. This model leads to hemiplegia, somatosensory impairment, motor deficits, and cognitive deficits. Our neurological exam scale enables us to evaluate all of these except cognitive function. Neurological exam was performed 48 h after the trauma was induced.

3.3.2 Induction of Diffuse Brain Injury with Weight-Drop Model

1. In general, experimental TBI models address cascades involved in brain injury which result from biomechanical impact. Four models are widely used in TBI research: (1) fluid percussion injury, (2) controlled cortical impact injury, (3) weight-drop (impact acceleration) injury, and (4) overpressure blast injury [15].
2. Marmarou's weight drop model was used to induce diffuse brain injury [16]. In brief, a trauma device, which works by dropping a constant weight from a specific height through a tube, was used [8, 11]. A metal cylinder weighing 300 g was dropped from a height of 1 m which induces a mild trauma as shown in Fig. 4. This quantity of weight has been shown to induce mild trauma in rats weighing between 250 and 350 g [17]. For the induction of trauma, the scalp of each anesthetized rat (ketamine 100 mg/kg and chlorpromazine 1 mg/kg IP) was shaved, a midline incision was performed, and the periosteum was retracted. In order to avoid skull fracture and to distribute the force homogenously, a stainless steel disc with

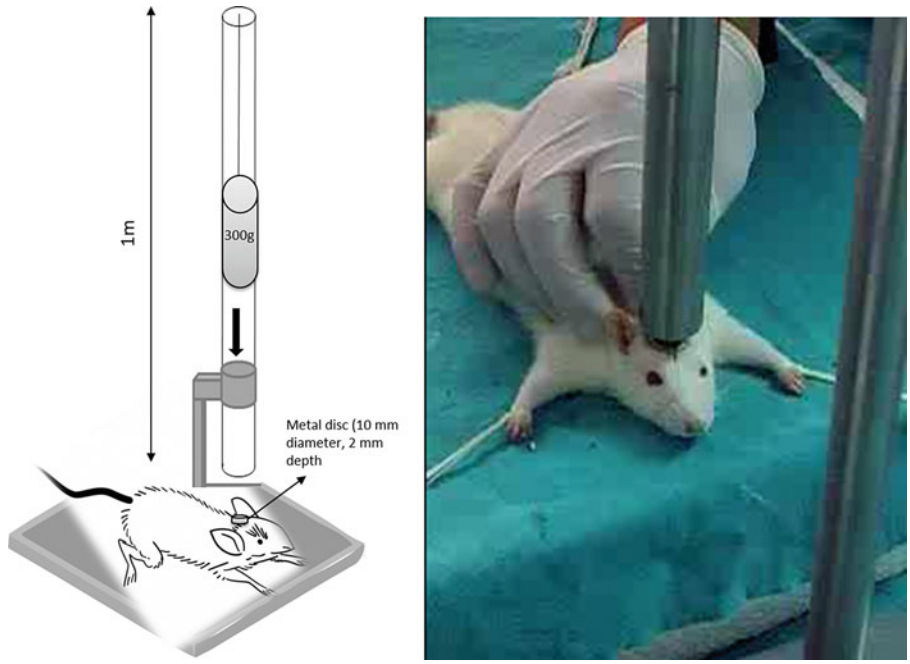

Fig. 4 Impact acceleration (weight-drop model): The diffuse brain injury was induced by dropping the freely falling 300 g steel from a height of 1 m above the skull

a diameter of 10 mm and a depth of 2 mm was fixed to the skull between the lambda and bregma using bone wax. Male Sprague Dawley rats (300–350 g) were placed in a prone position on a foam bed with a depth of 5 cm. The lower end of the metallic tube was then positioned directly above the disc (2 mm thick steel disk). The injury was induced by dropping the freely falling 300 g steel from a height of 1 m. An inflexible rope was tied to the weight to prevent repeated impacts.

3. This model leads to postural deficits, sensorimotor deficits, loss of sensation of nociceptive stimuli, and cognitive deficits. Our neurological exam scale enables us to evaluate of these all except cognitive function. A neurological exam was performed 48 h following the trauma.

3.3.3 Induction of Overpressure Blast Injury (OBI)

1. The TBI model was conducted by a shock tube to induce OBI in 2-month-old Sprague Dawley rats [12, 18, 19] (Fig. 5). Anesthetized rats (3% isoflurane) were placed into a polyvinyl chloride (PVC; 3 mm thick) holder exposing only their heads (body-armored setup). Their heads were placed 10 cm inside the open end of the shock tube (7 ft. in length; 4″ internal diameter).
2. Thus, the PVC holder positioned the specimen at 53 cm (20.87″) downstream from the section opening of the driven. Controlled overpressure is achieved by a 0.03 in. thick Mylar® membrane. The heads of the animals were positioned on a flexible mesh surface to diminish surface reflection of blast waves and decrease the formation of secondary waves that could potentially exacerbate the injury. Then the animals were subjected to a blast wave (30 psi peak pressure, 2–2.5 ms duration) directed at the animals lying below. Blast pressure was monitored with PicoScope data acquisition (#PS4227, Pico Technology, UK).
3. The shock tube produces a classic Friedlander waveform characterized by a positive pressure peak followed by a negative pressure peak. The neck, head, torso, and abdomen of the animal were fixed to the PVC animal holder in an effort to avoid movements which might cause tertiary blast effects. The peak pressure was recorded by a computer. A neurological exam was performed on the day of the sacrifice.
4. There were three groups: control (sham), single injury group (one time 30 psi peak pressure, 2–2.5 ms duration), and repeated injury group (30 psi peak pressure, 2–2.5 ms duration; on days 1, 4, and 7 during a week period). Rats receiving a single injury were sacrificed 1 week after the injury. Rats with repeated injuries were sacrificed on the eighth day of the repeated injuries [18].

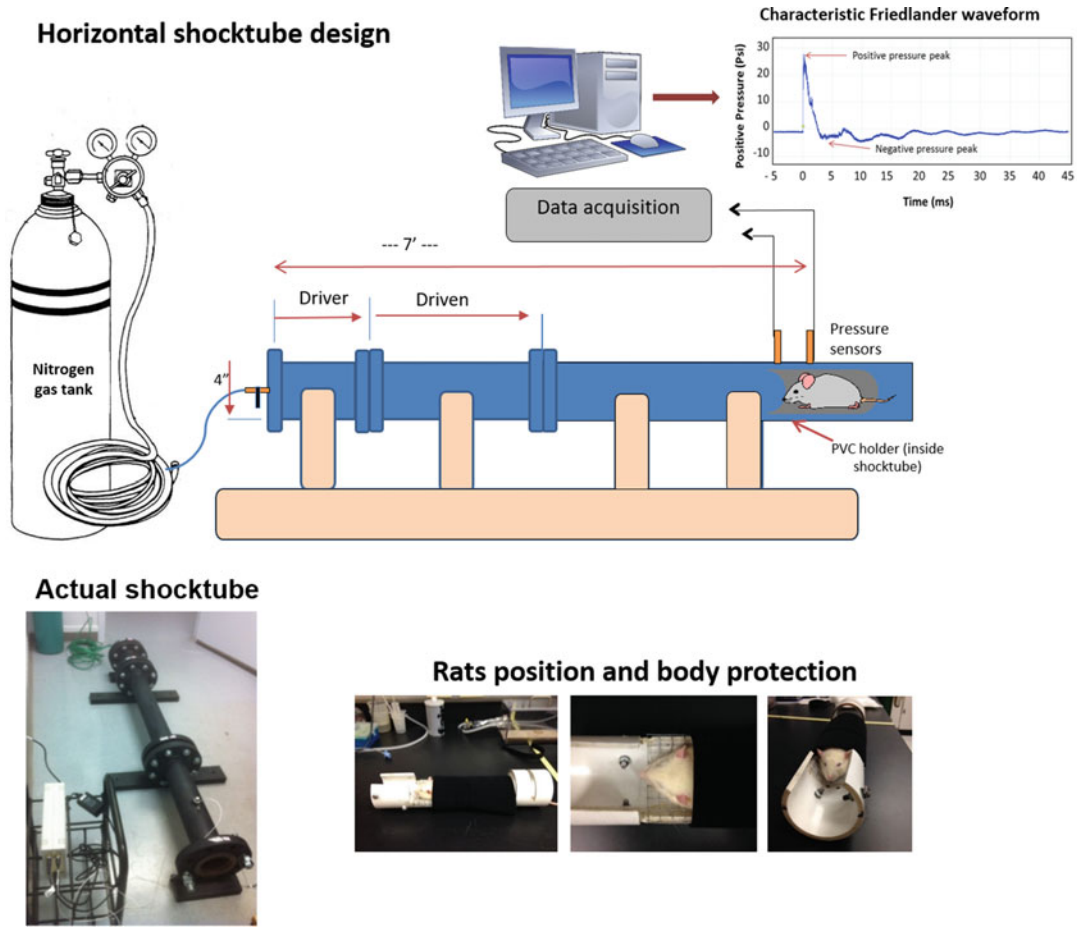

Fig. 5 Horizontal shock tube design for modeling overpressure blast wave-induced brain injury in rodents. Rats are placed in an animal holder made of 0.3-cm-thick PVC pipe that is placed 10 cm from the open end of the shock tube (*see* red arrow). The rat is inserted into the shock tube until the head is under the pressure sensor. The body is tightly wrapped with Velcro sticky back tape for fixation and to protect from the blast wave. Pressure was monitored with PicoScope data acquisition. Positive pressure peak occurs within a range of microseconds, while the first negative pressure peaks were observed at 3 ms (modified from [12, 19] with permission)

4 Results and Discussion

Although total scoring apparently demonstrates the difference between groups in all the models, there are discrepancies in the test components. For instance, changes in consciousness and lethargy are more pronounced in the subarachnoid hemorrhage model due to ischemic insult resulting from basilar artery vasospasm. Also, we observed decreased response to nociceptive stimulus, motor deficits (paresis/plegia), and altered response to climbing and rotation tests. Depending on the severity of the stroke, almost all components of our neurological test were affected. On the other

hand, with the other TBI models (weight drop and OBI), the cerebellar system was less affected. Therefore, the results from the rotation tests were not significantly altered in some rats.

Additionally, in clinical practice, it is difficult to reach a consensus as to which neurological exam to use to evaluate subarachnoid hemorrhage, closed head trauma, and cerebral ischemic diseases; therefore there are different grading systems for subarachnoid hemorrhage [20, 21]. Likewise, choosing a test and relying on a total score may not reflect the clinical manifestation completely in experimental models.

5 Conclusion

Toklu's modified neurological exam score method is a combination of balance, muscle strength, coordination, and reflex. Because of the easy setup and quick assessment, the scale can be used on a practical basis to assess neurological symptoms following traumatic brain injury and stroke. On the other hand, one must keep in mind that neurological exams with total scoring may be misleading in some cases. Brain injury is a combination of several mechanisms that involve neuronal death, brain edema, blood-brain barrier disruption, oxidative injury, metabolic dysfunction, and autonomic imbalance [22]. Because of the alterations in the autonomic nervous system activity due to diffuse brain injury [19, 22, 23], behavioral outcomes may not always correlate with histological changes. In other words, even though neuroprotection is achieved, this may not result in a significantly better neurological score. However, our observations with animal experiments demonstrate that an improved neurological score is associated with a higher survival rate and better prognosis in motor coordination [8–12]. On the other hand, following a stroke, we did not observe improved cognitive function in the short term (with passive avoidance test) despite better neurological scores achieved in our neurological exam scale [24].

While the analysis of motor function shows homology in primates and rodents, we suggest that Toklu's modified neurological exam test can be used to reflect clinical outcomes with the added advantages of easy setup and quick assessment. However, relying on a total score may not reflect the clinical manifestation completely. Given the fact that molecular recovery may not always be concomitant with clinical symptoms/outcomes, one must be cautious in terms of translational research. In order to accurately interpret results and draw conclusions, additional tests for cognitive and sensorimotor function may be required.

6 Notes

1. Having the observer blind to the treatment groups helps to eliminate bias. However, the experience of observer is also essential to standardize the scoring.
2. Stress may directly affect neurobehavioral experiments. Reducing the animal's stress and anxiety during testing is imperative to obtain reliable data. Therefore, handling the animals prior to testing is important to acclimate them to their new surroundings. In addition, testing the rats in their home cages versus testing them in new surroundings may affect the results.
3. In general, neurobehavioral analysis may result in a high variation among subjects. Therefore, using a small number of subjects may result in inconclusive results.
4. Depending on the severity of the trauma, a number of rats may die within the first few days. Therefore, it is important to design the experiments to use more rats based on the predicted mortality rates.

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