Journal of Clinical Research and Clinical Trials
2023 Volume 2, Issue 1
DOI: 10.59657/2837-7184.brs.23.005

BioRes Scientia
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Research Article

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Neurological Deficit Methodical Approaches to Experimental Study

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Abstract

Freasibility of studying pathology of the brain in the experiment leeds to the need for adequate ways to assess the neurological deficit that occurs in animals, including sensorimotor and behavioral disorders, as well as disorders of higher nervous activity (memory, gnosis, praxis, emotional behavior, etc.). The rat is one of the adequate objects of experimental research to study brain disorders in various pathologies. The review summarizes the literature data on possible ways to evaluate sensory-motor reflexes, learning ability and memory in experimental animals at different ages. In the early postnatal period, reflexes are evaluated: "flipping on the plane", "negative geotaxis", "avoidance of breakage", "acoustic stimulus response", "olfactory reaction" and "muscle strength". To study the degree of neurological and behavioral disorders in adult animals, a number of methods can be used: the Bederson test, the test for evaluating modified indicators of the depth of neurological deficit, the Garcia test, the angular test, the paw pulling test, the "open field" test.

Keywords: neurological deficit; methodical approaches; experiment

Introduction

Freasibility of studying pathology of the brain in the experiment leeds to the need for adequate ways to assess the neurological deficit that occurs in animals, including sensorimotor and behavioral disorders, as well as disorders of higher nervous activity (memory, gnosis, praxis, emotional behavior, etc.). The rat is one of the adequate objects of experimental research to study brain disorders in various pathologies [1].

The review summarizes the literature data on possible ways to evaluate sensory-motor reflexes, learning ability and memory in experimental animals at different ages. In the early postnatal period, reflexes are evaluated: "flipping on the plane", "negative geotaxis", "avoidance of breakage", "acoustic stimulus response", "olfactory reaction" and "muscle strength". To study the degree of neurological and behavioral disorders in adult animals, a number of methods can be used: the Bederson test, the test for evaluating modified indicators of the depth of neurological deficit, the Garcia test, the anglular test, the paw pulling test, the "open field" test.

Before the study, a number of conditions must be met: animals are placed in a quiet, low light place at least 60 minutes before testing, regrouping of animals, their feeding and other active manipulations are excluded in order to reduce the error. Administration of pharmacologic agents, which are necessary during the experiment should be carried out in the most standard way. Tagging of animals, transplantation into other cells, the enrolling of new groups should be carried out no less than a day before the conduct of behavioral tests. In order to prevent mother rats from eating their offspring, all manipulations with newborn baby rats are performed no earlier than two days after birth, and only with gloves [2].

Tests to study the maturation of the nervous system during the newborn period

In newborn baby rats, methods for evaluate the development of the nervous system can be used to assess the rate of appearance of sensory-motor reflexes ("flipping on the plane", "negative geotaxis", "avoidance of breakage", "acoustic stimulus response", "olfactory reaction" and "muscle strength"). They can be carried out both in dynamics and once, on the expected day of reflex maturation in intact animals [3].

To assess the ability to flip on the plane, starting from the 2nd day of postnatal development, the baby rats are placed on their backs and the time required to return to the starting position is measured. The formation of the reflex is considered complete if the baby rats are able to turn over on their stomach (on

average – on the 8th day of postnatal development). The experiment is carried out with each animal for 30 seconds.

From the 5th day of postnatal development, the "negative geotaxis" reflex is examined. The study is conducted **once a day**, for **1** minute. The baby rats are placed head down on a plane with an inclination of 25 °. By the 7th day, the animal must be able to turn 180° [4].

From the 6th day of life, the "cliff avoidance" reflex is formed. To study it, the baby rats are placed on a table or a raised platform so that the front paws of the animal hang a little from the edge of the table or platform. The reflex is formed by the 9th day after birth. At this age, the baby rats crawl out from the cliff. The time for which the animal must crawl out does not exceed 10 seconds.

On the 6th-8th day after birth, a "pendulum reflex" is formed. It consists in changing the direction of the animal's head and body at a right angle in a horizontal plane with the help of the front paws, while the hind limbs remain pursed and stationary. The number of turns in 60 seconds and the number of reversals (changes in the direction of movement in the opposite direction) are calculated.

The "acoustic stimulus response " is determined from the 8th day of the postnatal period, but it is fully formed only by the 13th day of life. The baby rats are placed on an elevated platform in a cage with sound insulation. It is applied to an acoustic stimulus lasting less than 1 second. The rat's movement is tracked in response to the sound [5].

On the 10th-11th day, the reflex "olfactory reaction" is examined. A bar with divisions is placed on the cells and a rat is placed on it. The distance at which the animal correctly chooses the direction to the cage with the rats in which it was located before the experiment is recorded. Take into account the number of falls and errors in the direction of movement. About14 days of development in a dark room, the "pupillary reflex" is determined. The light source is point. Registration is subject to turning the head of the rat or changing the width of the pupil of the animal.

The “muscle strength” reflex is assessed from the 15th day of postnatal development. To study it, the rat is placed on a fine metal grid, which is then slowly turned over. The length of stay of the rat under the bars is noted. The time spent under the grate is usually about 15 seconds.

Figure 1: Assessment of the "muscle strength" reflex.

Experiments are conducted until the final formation of reflexes in all broods of intact animals. To study emotional-motor behavior and the ability to fine motor coordination, the following tests are used: “turning over in free fall”, “holding on a rotating cylinder”, “spontaneous motor activity”.

The ability to roll over in free fall is examined on the 17-20th day after birth. Animals are held upside down on a soft surface and then let it out of hands. The drop height is about 0.5 meters. Assess the ability of the animal to roll over in the air so as to fall on all fours.

From the 14th to the 25th day of life, the ability of rat pups to stay on a rotating cylinder with a rubber surface with a diameter of approximately 12 cm at a rotation speed of 30 rpm is studied. By the 25th day after birth, the rat should be kept on the cylinder for at least three minutes. The task can be complicated by changing the size of the cylinder, the material of its surface and the speed of movement.

"Spontaneous motor activity" can be investigated on the 30-45th day in a variety of ways: using the "squirrel wheel", and other devices with electronic or magnetic registration of activity. Studies are carried out until the skill is fully formed in all control broods. [6]

Tests to examine sensorimotor disorders in adult animals: To examine the degree of sensorimotor disorders in adult rats, a variety of methods are employed: the Bederson test, the modified neurological deficit depth test, the Garcia test, the angle test, the paw stretch test, the open field test.

The Bederson test is as follows: The rat is held by the tail, at a distance of 1 meter above the floor, and the mobility of the forelimbs is observed. Normally, rats pull their limbs towards the floor. The test contains placing the rats on a slippery, smooth surface and applying gentle pressure from the side behind the shoulder until the forelimbs begin to slide. Animals must resist sliding equally in both directions. [7]

Neurological depth assessment scale: This scale includes tests to detect motor activity during animal tail suspension, features of walking on horizontal plane, coordination of movements when walking on the beam, the severity of reflexes (Pinna reflex - when touching the external auditory meatus, the rat should shake its head; corneal reflex - the animal blinks when touching the cornea of his eye). [8].

The Garcia tests: Includes assessment of spontaneous activity in the cage for 5 min, the symmetry of forelimb extension when hanging animals by the tail, the ability to climb the wall of the lattice cage, the response to touching each side of the rat's body, and the response to touching vibrissae.

Angle test: This test allows you to evaluate disorders of perception of space and gaze paresis. The rat is placed between two vertical planes. Intact rats easily turn both to the right and to the left. With a number of pathologies, including cerebral ischemia, a neglect is observed - while the animal is not able to perceive a certain part of the space.

The "paw stretching" test: Allows to identify and evaluate disorders of the motor activity of the forelimbs. The limbs of the rat during the study should hang down without support, then it is raised to the edge of the platform so that its vibrissae touch the surface of the plane. The animal is held by the hands and pulled sideways over a smooth surface. The number of movements of the forelimbs performed on the side from which the rat was pushed is recorded. Intact rats at the same time make a lot of movements with their front paws. [9]

Open field test: This test was proposed to record the behavior of animals in response to "new, potentially dangerous stimuli." It is played on a flat surface, marked with lines forming 36 squares of the same size and fenced around the perimeter. Usually, in the "open field" they study the time of exit from the center of the site, where the rat is placed at the beginning, activity in the horizontal and vertical planes of space, grooming (washing), the study of deepening and holes by the animal, and defecation. In the "open field" you can observe violations of the motor sphere, for example, register discoordination, trembling, paresis, paralysis. Horizontal motor activity of animals includes running in different directions, walking in a circle. At the same time, participation in the movements of all the limbs of the rat is evaluated. One crossed sector is taken as a unit of movement during visual registration of activity. [10].

The motor activity of rats in the vertical plane is represented by two forms of racks. Climbing – the hind legs of the animal remain on the floor of the surface, and the front ones bear against the wall of the "open field", and rearing – if the forelimbs remain on the weight. Grooming is divided into short and long. Short grooming is rapid circular movements of the front paws around the nose and vibrissae, and long–term grooming is washing the eyes, the area behind the ears, washing the entire head, paws, sides, back, anogenital area, tail. Researching of ports in the floor consists in sniffing their edges or thrusting the muzzle inside the holes. The number of acts of defecation is considered an index of the "emotionality" of the animal.

Methods based on the development of conditioned reflexes are used to study the cognitive abilities of animals. The most complex functions of the brain are cognitive. It provides rational cognition of the surrounding world and purposeful interaction with it is carried out. This process includes the following components: perception of information (gnosis),

processing and analysis of information (arbitrary attention, generalization, identification of similarities and differences, establishment of associative connections), memorization and storage of information (memory), information exchange, construction and implementation of an action program (skills). [11].

Passive avoidance with negative (painful) reinforcement: Involves assessing the memory of animals. The method is based on the natural tendency of rats to avoid illuminated space. Animals are placed in a lighted cage. On the first day of training, rats are placed in a dark cell and get electrocuted. A day later, the time spent by the animals in the illuminated space is examined about two minutes.

The number of rats that do not avoid the light chamber, the time of transition to the dark chamber at the first pain irritation and a day after the start of the experiment are recorded. [12]

Active avoidance with negative (painful) reinforcement: It is examined in a cage divided by a vertical partition into two compartments. In the floor of the cage there are grids through which an electric current is supplied, the strength of which is individually selected for each rat. The formation of the skill begins with the presentation of audio or visual signal. If after the tone the rat does not leave the dangerous place for ten seconds, it receives an electric shock. The experiment is completed after 80% of intact animals have formed a skill. Successful training is considered if in 20 cases the rat has avoided pain irritation 18 times. During the study of long-term memory, rats are not supported by pain, but the extinction of the skill is investigated for 10 days. They record the average number of correct answers depending on the number of presentations, the learning curve (the relative number of animals that have achieved stable skill formation with a given number of tests), the average value of the pain threshold, the percentage of erroneous answers, the rate of skill extinction. [13]

Maze learning with positive (food) reinforcement: For this test, T-, V-shaped or cross-shaped mazes are used (Figure 2). The mass of rats is measured before limiting their diet, after the first day of the experiment and after the completion of the training process. The average number of correct answers, depending on the number of presentations, the emotional reaction of a labyrinth, the loss of body weight during training and correlation of this indicator with the rate of skill acquisition, the number of visits to various compartments of the maze, the time spent in them, the period of motor activity and the period of immobility in open or closed sectors of space, the length of the path traversed in the maze are recorded, the average, maximum and minimum speed at which the rat moved through the compartments, the number of turns and circles in place, activity in the vertical and horizontal planes.[14]

The experiments are continued until at least 80% of the control group animals reach the training criterion. In the absence of differences in learning speed, the memory of trained rats is examined. At the same time, it is necessary that the rats receive a similar amount of reinforcements during the training process. [15]

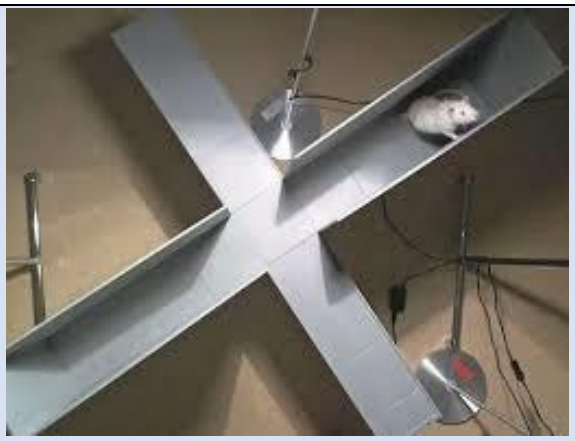

Figure 2: A raised cross-shaped maze for studying the behavioral activity of rats (top view).

That way, there is an extensive range of methods for assessing neurological disorders in laboratory animals, which makes it possible to study the level of development of neurological deficits in various pathologies of the nervous system, including cerebral ischemia, as well as to study disorders of brain maturation in postnatal ontogenesis when modeling embryonic and fetopathies and testing ways to correct them.

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Cite this article: Maksimovich N. Ye, Bon E.I, B. Vishnevskaya L.I, Maroz K.V. (2023). Neurological Deficit Methodical Approaches to Experimental Study. *Journal of Clinical Research and Clinical Trials*, BRS Publishers. 2(1); DOI: 10.59657/2837-7184.brs.23.005

Article History: **Received:** February 12, 2023; **Accepted:** March 13, 2023; **Published:** March 20, 2023