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Review

Behavioral Tests Used in Experimental Animal Models

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Abstract

Experimental animals are used to develop treatments for diseases in humans and animals and to control pathophysiological processes. Biochemical and pathological parameters are still insufficient to explain behavioral disorders. Therefore, behavioral models are necessary to understand the pathophysiology of diseases and accelerate the development of treatments. Experimental animal models show the clinical reflection of many purposes such as drug research for diseases, the toxicity of drugs and prevention of toxicity, understanding drug effects, and understanding of biological processes in diseases. Although data from behavioral models can be evaluated using a single test, it is more efficient to use a set of tests consisting of different tests that show traits on the behavioral phenotype. In this study, elevated plus maze, passive avoidence, locomotor activity, fear conditioning, morris water maze, randall selitto paw pressure, von frey, forced swim and, apomorphine-induced rotation tests, which are widely used in neurological studies in mouse and rat species, are explained. In addition, it is mentioned for what purpose the specified behavior models are used. In this review, important behavioral models commonly used in neurological studies measure which parameter, their procedures, and goals, and some of the animal- and environmental-related factors that affect behavior.

Keywords: Behavioral test, Neurodegeneration, Animal model, Anxiety, Depression

1. Introduction

Since the early 1900s, animal models have been frequently used in experimental studies for the study of biological processes and the understanding of diseases. With the increase in research opportunities and the development of reliable test systems, the use of animal models has also increased [1, 2].

Experimental animal models are used for many purposes such as drug research for diseases, toxicity of drugs and prevention of the toxicity, understanding drug effects, and understanding biological processes in diseases [3-6]. There are changes in the behavior of humans and animals due to neurodegenerative diseases, pain, and damage to various tissues. For this reason, behavioral tests used to measure behavioral changes have an important place in studies using experimental animals [7]. This review focuses on the use of important behavioral tests commonly used in neurological studies in mice and rats in experimental animal models.

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2. Behavioral Tests

Experimental animals are used to develop treatments against diseases and to control pathophysiological processes. Behavioral tests are used to measure changes in disease parameters [8, 9].

The use of behavioral tests is controversial because it is not known exactly whether an experimental animal can think like a human. In addition, it is thought that the diseases covered by neuroscience can be understood by evaluating only biochemical or pathological changes [10]. However, it should not be forgotten that in neuroscience, it is necessary to focus on both cognition and mood to understand animal and human behavior [11]. However, it should be kept in mind that animal experiments are an artificial imitations and biological behavior may be different in humans. In this context, the correct interpretation of animal behavior is the key point of preclinical studies in order to accurately evaluate the reflections of neurodegenerative disorders in human models using experimental animals [12].

2.1. Factors Affecting Behavior in Tests

Behavioral tests show differences depending on the physiological characteristics of the animal, such as species, sex, age, and/or environmental characteristics, as well as pathological disease states. For this purpose, tests are carried out as much as possible by comparing them with control groups. Data obtained from control groups in studies conducted in different laboratories cannot provide a complete reference because the physiological characteristics or environmental factors of experimental animals are different [13, 14].

2.1.1. Factors Related to Animals

The ability of animals used in behavioral models to give the most optimal results depends on the correct execution of the experiment, and animal-related factors have a major place in this regard. Inaccurate measurements may be encountered depending on the errors made in the grouping of animals [15]. Housing animals in groups is a principal factor for them to get away from the stress of social isolation. On the other hand, keeping experimental animals overcrowded cages causes stress in animals. For this reason, it is not recommended to keep animals in cages alone or in crowds of more than five animals. Another mistake made in the grouping is that these animals tend to colonize based on sex. Male rats in cages can compete against each other for superiority. As a normal behavior, they can fight with each other, in which case injuries or deaths can be seen. As a result of the normal process, aggression, anxiety, and depression-like behavioral disorders may be encountered in some animals. Therefore, animals should be followed regularly [16].

Depending on the species, differences can be seen in the activities, self-interest, and cognitive performance of experimental animals. For example, B6 mice show higher self-care and cognitive performance in locomotor activity results and lower anxiety levels compared to 129 mice [13]. Age is an important parameter of the behavior of experimental animals. The cognitive performance of animals generally decreases as they get older [17]. In addition, the separation of animals from the mother in the early postnatal period affects behavioral parameters. It has been reported that deprivation of the mother during this period increases the period of inactivity and decreases the climbing time [18].

2.1.2. Environmental Factors

The environment is an important parameter for experimental animals, and it is recommended to provide environmental enrichment of experimental animals in international guidelines. The cognition and learning of experimental animals may change depending on the environment they live in [14]. Many studies have shown that improving environmental conditions in experimental animals such as rats and mice has positive effects on learning and memory, neuronal cell size, synaptic size, dendritic branching, and neurogenesis [14, 17, 19].

Environmental changes can lead to developmental effects through epigenetic factors and cause changes in the behavior of animals [13]. Experiences in the early stages of life make a significant contribution to the behavioral development of experimental animals. It has been determined that environmental enrichment in the first trimester after birth causes an increase in learning and exploration behavior and a decrease in anxiety in experimental animals at later ages [14, 20]. In addition, food restriction causes an increase in immobility time in experimental animals. In studies, it has been determined that animals exposed to such situations show behaviors similar to depression and anxiety [21]. Exposure of experimental animals to stress factors such as hunger and cold in the environment they live in is another issue that affects their behavior. The incidence of depression increases in animals living in an environment dominated by stress factors [22]. Similarly, depressive behaviors are observed in the offspring of animals exposed to prenatal stress. Therefore, stressful conditions should be eliminated as much as possible in a laboratory used for behavioral testing [23].

3. Use of Behavioral Tests in Experimental Animal Models

Behavior is shaped through connections between regions of the nervous system [24]. A change in the central nervous system, interregional interaction, or pathology in any part of the body may cause behavioral actions to occur. For example, behavioral changes in Alzheimer's disease are caused by damage to the relevant brain regions, while behavioral changes in patients with stroke are usually due to changes in areas far from the damaged area of the brain. Behavioral changes in conditions such as hepatic encephalopathy and diabetic neuropathy occur due to disorders in different organs [24-26]. Although data from behavioral models can be evaluated using a single test, it is more efficient to use a set of tests consisting of different tests that show traits on the behavioral phenotype. Data obtained using different tests can be used to create derived variables. Such variables give a more comprehensive result than individual behavior profiles [27].

3.1. Elevated Plus Maze (EPM) Test

The EPM test is a test used to measure unconditioned responses of anxiety. EPM is a behavioral model that does not require the training of animals and has high ethological validity [27]. This test, which was first developed by Handley and Mithani, is frequently used in the assessment of anxiety [28]. Following the development of the test, researchers recognized in 1986 that it was indeed a test capable of assessing anxiety.

The EPM test is still actively used in the measurement of anxiety [29].

The apparatus used in this test stands higher than the ground and has two open and two closed arms. The behavior of experimental animals to avoid unprotected, bright and high places is measured with this designed mechanism. At the same time, with this test, the willingness of experimental animals to explore new environments can also be evaluated. The behavior of avoiding open environments in the EPM test indicates avoidance of physiological stress conditions, and if the animal is confined to this environment, corticosterone levels and defecations increase in relation to anxiety. If anxiolytic drugs are given to the animals in this test, their desire to explore the open arms of the labyrinth increases [29].

In the test, the time spent by the animals in the closed arm and the open arm reveals the state of anxiety, while the number of entrances to the arms and the spontaneous motor activities of the animals are measured. In addition, ethological criteria such as the number of head dipping, dropping of stools, and frozen or tense postures can also be evaluated in this test (Figure 1) [30].

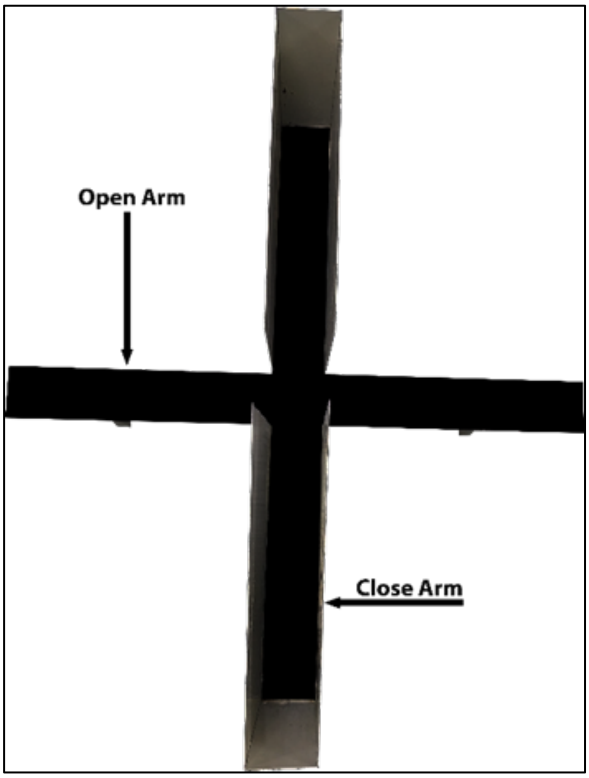

Figure 1. Elevated Plus Maze Test Device

3.2. Passive Avoidance (PA) Test

Unlike the EPM test, the PA test is used to measure conditioned responses to anxiety. In this test, animals are trained before the experiment, and they learn the test setup with the help of a trigger such as an electric current [27]. As a result of the PA test, data on anxiety-related behavior, learning and memory parameters are obtained [31].

Generally, nocturnal animals like rats and mice are used in the studies. Behavior in nocturnal animals is sleeping during the day and being active at night, and these animals are disturbed by light. That's why nocturnal animals generally tend to spend time in dark environments. The PA test setup consists of two rooms, light, and dark. While the bright room is covered with high lumen led lamps, the dark room contains metal rods that give electric current to the animals. Normally, animals tend to switch to dark environments because they are uncomfortable in high-lumen light environments. However, if animals go into the dark environment, they are exposed to an aversive electric current, and by repeated measurements, the subjects learn the system. It is expected that animals, which naturally tend to switch to the dark room, do not remember the electric current [31-33].

Measurements are at 5 minutes and animals undergo a 10 second acclimatization period. After the acclimation period, the bright compartment is exposed to high-lumen light and the door to the dark compartment is opened. If the experimental animal enters the dark compartment, the door is automatically closed, and an electric current is applied to the animal. In this setup, the animals are measured on two consecutive days as initial and step-through. In repeated tests, the prolongation of the time that the experimental animals spent in the light area is interpreted as they learned that they were exposed to electric current in the dark room (Figure 2) [34, 35].

Figure 2. Passive Avoidence Test Device

3.3. Locomotor Activity (LA) Test

The LA test is used to measure motor activity in experimental animals. This test is frequently used in the detection of cases related to impaired motor functions and in cases of anxiety [36]. Rather than a conditional or unconditional measure of anxiety, as in the EPM test or PA test, this test focuses on the change in the motor activities of animals in the state of anxiety [36, 37].

Square shaped transparent meshes are used in the LA test. After the experimental animal is placed in the cage, the system is started. This system, which is connected to the computer, scans with infrared rays and automatically measures the distance, stereotypical, resting, horizontal, vertical, and ambulatory movements of the experimental animal without any external intervention. Based on these data, comments about the curiosity, adaptation to a new environment, and anxiety of experimental animals are obtained (Figure 3) [36]. The stereotypical movement measured in the test is the recognition of the environment and is also considered grooming. Horizontal movement is the movement of the experimental animal on the ground without standing on its hind legs. Vertical movement is the movement of standing on the hind legs. Ambulatory movement is the displacement of the experimental animal without standing up. Distance is the movement distance of the experimental animal. Resting is a parameter measured depending on the resting time of the experimental animal. The sum of horizontal, vertical, and ambulatory movements is considered as total LA [37].

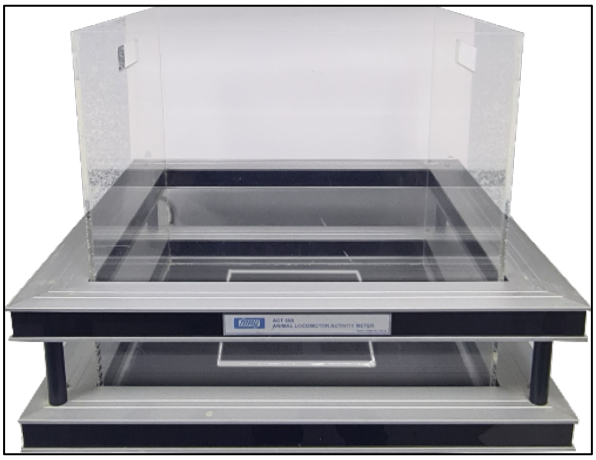

Figure 3. Locomotor Activity Test Device

3.4. Fear Conditioning (FC) Test

Fear plays an important role directly or indirectly with psychiatric conditions [38]. Fear is a normal emotion in most animal species, from experimental animals to humans, and is an anxiety response to danger. In cases of anxiety, fear appears persistently and irrationally, to such an extent that it interferes with daily life [39].

FC is a behavioral test that reveals behavioral and physiological response with high reliability, including learning and measurable data under the control of the experimenter [38]. This test is rooted in Pavlovian aversive conditioning. In performing the test, repeated pairings to an unpleasant neutral stimulus such as tone of voice, shape, light, and pain are performed, and conditioned fear is acquired. As a result, the experimental animal can give great reactions to the neutral stimulus and are afraid. In cases of fear-related psychiatric illness such as anxiety, animals can give greater responses to stimuli, and the fears of animals with successful treatment remain at a basic level (Figure 4) [40]. Anxiolytic drug application to experimental animals after the FC test affects the formation of conditional learning and the data obtained as a result of the experiment is at a lower level. In addition, the FC test has been associated with other anxiety-related psychiatric disorders such as panic disorder, agoraphobia, and post-traumatic stress disorder [41].

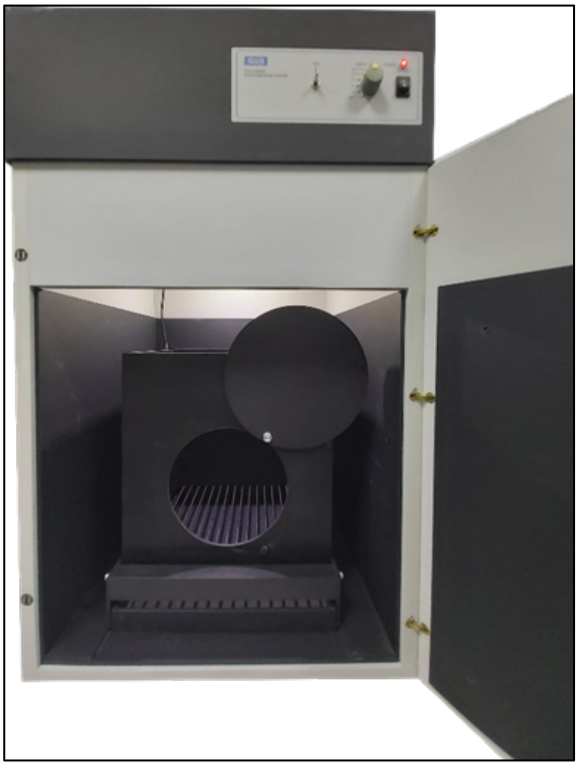

Figure 4. Fear Conditioning Test Device

3.5. Morris Water Maze (MWM) Test

Spatial intelligence is a very important ability in terms of color, shape, size, location, and relationships between animals. Spatial intelligence, which integrates the mind and the environment, organizes the shapes and objects in the environment in the mental dimension. Learning and memory abilities, which are associated with spatial intelligence, affect many behaviors from finding mates to finding shelter and food, and are one of the basic skills necessary for survival [42-45].

Experimental animals are conducive to learning new things and keeping them in their memory. The MWM test is a frequently used test for learning and memory experiments of experimental animals. In this test, the spatial intelligence, maze solving skills and memory of animals are measured [42, 43].

Although experimental animals can swim from birth, they generally dislike swimming and tend to avoid water [42]. Animals that do not like water and tend to escape from the water as soon as possible try to solve the connection between the shapes around the pool and the platform at one point of the pool. The MWM test takes 6 days. On the first day, they are left in the pool without a platform for 60 seconds to get used to the water. With the MWM test, measurements are made from different parts of the pool for 4 days, so that the platform is embedded in the memory of the experimental animals and their learning skills are improved. The test is performed with 90-second measurements, and experimental animals that cannot find the platform for 90 seconds are considered unsuccessful in this test. On the sixth day, the platform is removed from the pool and the time spent by the experimental animals in the target quadrant for 60 seconds is determined. Prolonged time spent in the target quadrant indicates good memory and cognition. As a result of the MWM test, the experimental animals recognize the pool in 3 dimensions. In repeated measurements in healthy animals, the determination time of the apparatus is shorter. This shows the positive situation on learning and cognition (Figure 5) [46-50].

In disease models that directly affect memory, such as Alzheimer's disease, the time for experimental animals to find the platform in the MWM test is longer. In the test, which is evaluated with baseline and post-experimental measurements, experimental animals that solve the platform in basal measurements cannot remember or re-solve the platform they initially solved in case of a disease affecting learning memory. When compared with the control groups, the diseased groups give unsuccessful results in this test and the treatment can be evaluated according to the success of this test [48-50].

Figure 5. Morris Water Maze Test Pool

3.6. Randall-Selitto Paw Pressure (RSPP) Test

Neuropathic pain has been described as the worst of all suffering that a neural injury can cause. This type of pain is characterized by dysesthesia, hyperalgesia, and allodynia and is seen in cancer, spinal injury, multiple sclerosis, and stroke patients [51].

Tests used to measure pain are categorized as reflexive and non-reflexive. Reflexive tests are also divided into mechanical, electrical, hot and cold stimuli. The RSPP device is a measurement technique used to detect reflexive mechanical hyperalgesia. In this test, the animal's paw is placed in a Randall-Selitto analgesiameter and the device applies a certain pressure to the paw. When a withdrawal reflex or sound formation is observed in the animal, the test is stopped, and the final pressure applied is evaluated as the pain threshold. The RSPP test finds use in many types of pain such as inflammatory, neuropathic, arthritis-related, muscular, and cancer-related (Figure 6) [52].

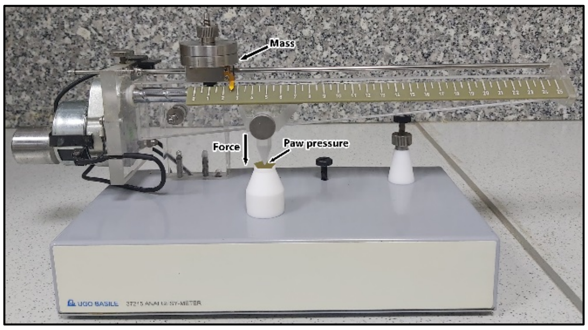

Figure 6. Randall-Selitto Analgesiameter

3.7. Von Frey (VF) Test

The VF test is a technique used to measure the mechanical allodynia of pain. The nociceptive threshold assessment performed in this test is largely similar to the pain sensitivity tests performed in humans [53]. A plate with a square hole in the bottom is used to perform the VF test, and the experimental animal is placed in a transparent box on this plate. Mechanical allodynia is evaluated as the sequential application of filaments with different bending strengths. The filaments, which are applied in order according to their thickness, are gently held on the paw of the experimental animal 5 times for 5 seconds. In the VF test, claw retraction responses are evaluated by detecting which filament the animals last rely on. In this measurement, it is determined that the less the animals react to the pain, the more they are sensitive to pain. In performing the VF test, care should be taken to ensure that the animals are alert, not grooming, and that their paws are in contact with the platform (Figure 7) [54].

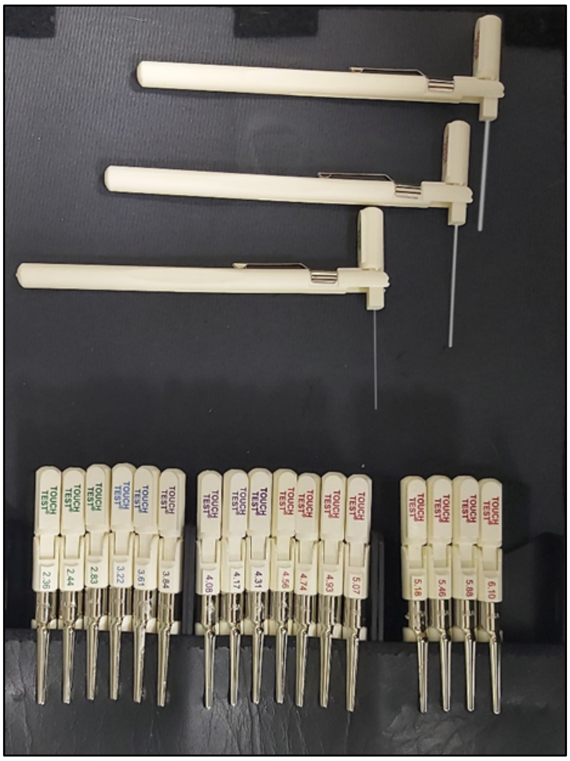

Figure 7. Von Frey Test Flaments

3.8. Forced Swim (FS) Test

The FS test is a successful behavioral test used to understand depression. In this test, behavioral hopelessness and changes in motor activities are measured depending on parameters such as fatigue and energy loss of animals [55, 56].

In the FS test, also known as the Porsolt test, the experimental animal is placed in a cylindrical container filled with water from which it cannot escape [57]. After the experimental animal tries the first fighting, swimming and climbing activities in this container, it eventually starts swimming or remains motionless. While it is thought that there is a depression-like effect in immobile animals, it is assumed that active animals are not depressed. It is a valuable test in new drug development because the depressive behavior observed in the FS test can be eliminated with almost all antidepressants. At the same time, while antidepressants are effective in long-term use in humans, they can give acute results in the FS test [58].

In carrying out the FS test, animals are measured for 5 minutes. Tests can be performed basal or post-treatment as two measurements. Experimental animals are kept in water for 15 minutes to acclimate on the first day. On the second day, the test is performed by taking 5-minute measurements in the FS test. In the video recordings taken as a result of the test, if the animals are swimming motionlessly with their noses above the water, they are called "still". If there is an action of swimming in such a way that the forelimbs of the animals break the water surface, it is called "fighting". The observation of rowing movements in the front and hind limbs of animals is called "swimming". The evaluation of experimental animals is shaped according to the time they make these movements (Figure 8) [55].

Figure 8. Forced Swim Test

3.9. Apomorphine Induced Rotation (AIR) Test

Apomorphine is a dopamine receptor agonist that acts non-selectively on dopamine receptors. Administration of apomorphine to experimental animals causes excessive stimulation of D1 and D2 dopamine receptors due to damage in the striatum region of the brain [59]. In case of damage to the striatum region of the brain, experimental animals perform a contralateral rotational movement in the opposite direction of the lesioned region [60].

The AIR test is used to verify the model and test the efficacy of treatment in animals modeled on the disease, Parkinson's disease, which damages the striatum region [60]. However, the method can detect damage in cases where dopamine is completely lost. Therefore, it is more useful in the evaluation of advanced Parkinson's disease rather than mild and early-stage Parkinson's disease [59].

In performing the AIR test, the subjects are placed in a square-shaped cage and left for a while to get used to it. After the acclimatization period, apomorphine injection is given to the experimental animals. Experimental animals that start to rotate after apomorphine administration are recorded and the number of 360-degree contralateral rotations is calculated [61].

4. Conclusions

Neuroscience is evolving and developing useful behavioral models is a challenge. Biochemical and pathological parameters are still insufficient to explain behavioral disorders. Therefore, behavioral models are necessary to understand the pathophysiology of diseases and accelerate the development of treatments.

The number of available animal models has increased so much today that model selection has become difficult for experiments. The choice of behavior model for the experiment is made as a result of parameters such as what is to be measured, the procedure and design of the test, and the limitations of the model. It should also be known that external factors affecting behavior parameters should be kept at a minimum level as much as possible. A false positive or false negative measurement causes erroneous results and creates scientific data pollution.

The parameters, procedures and objectives of the important behavioral models commonly used in neurological studies are discussed in this review. In addition, some of the animal and environment-related factors affecting behavior are mentioned in this review. Although measurement processes are difficult and require significant infrastructure support, behavioral tests are invaluable test systems for measuring behavior-related diseases, developing new drugs and observing the clinical reflection of data.

Conflict of Interest

The authors declare no conflict of interest.

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