Invited Review / Davetli Derleme
doi: 10.18678/dtfd.1493348

Duzce Med J, 2024;26(S1):65-71
Düzce Tıp Fak Derg, 2024;26(S1):65-71

Animal Experiments Used in Experimental Neuroscience Research: Learning, Memory, Anxiety, Depression and Motor Function Behavioural Experiments

Deneysel Sinirbilim Araştırmalarında Kullanılan Hayvan Deneyleri: Öğrenme, Hafıza, Anksiyete, Depresyon ve Motor Fonksiyon Davranış Deneyleri

Güven AKÇAY
0000-0003-3418-8825

Department of Biophysics, Bolu Abant İzzet Baysal University Faculty of Medicine, Bolu, Türkiye

ABSTRACT

Behavioral experiments have been conducted since the classical conditioning research of Ivan Pavlov in 1904. Experimental research plays an important role in understanding the mechanisms of diseases, preventing these diseases, and developing effective treatment methods. Research using animal models is very important to understand the mechanisms of these diseases and to develop effective treatment strategies. Animal models are widely used in the research of a treatment method, the development of novel treatment protocols, and the discovery of new drug molecules. The efficacy of the drug to be developed is very important both for testing whether the animal model is formed before starting the research and for the effectiveness of the drug in treatment and for the elucidation of the mechanisms to be investigated. Therefore, evaluations are usually made with behavioral experiments. Each behavioral experiment has its own advantages and disadvantages. Therefore, the researcher should be aware of these advantages and limitations before choosing the most appropriate behavioral experiment. This review aimed to describe the most commonly used learning, memory, anxiety, depression, and motor function behavioral experimental protocols in experimental models such as Alzheimer's, epilepsy, migraine, neuropathic pain, schizophrenia, Parkinson's, cerebral ischemia, and traumatic brain injury.
Keywords: Anxiety; depression; learning; memory; motor function.

ÖZ

Davranış deneyleri, 1904'te Ivan Pavlov'un klasik koşullanma araştırmalarından beri yapılmaktadır. Hastalıkların mekanizmalarının anlaşılması, bu hastalıkların önlenmesi ve etkin tedavi yöntemlerinin geliştirilmesinde, deneysel çalışmalar önemli rol oynar. Hayvan modelleri kullanılarak yapılan araştırmalar, bu hastalıkların mekanizmalarını aydınlatabilmek ve etkin tedavi stratejileri geliştirebilmek için oldukça önemlidir. Bir tedavi yönteminin araştırılmasında, yeni tedavi protokollerinin geliştirilmesinde ve yeni ilaç moleküllerinin keşfedilmesinde, hayvan modelleri yaygın olarak kullanılır. Geliştirilecek olan ilacın etkinliği hem araştırılmaya başlanmadan önce hayvan modelinin oluşup oluşmadığının test edilmesi için hem de ilacın tedavideki etkinliği açısından ve hem de araştırılacak mekanizmaların aydınlatılması için oldukça önemlidir. Bundan dolayı genellikle davranış deneyleri ile değerlendirmeler yapılır. Her davranış deneyinin kendine özgü avantajları ve dezavantajları vardır. Bu nedenle araştırmacı, davranış deneyini seçmeden önce bu avantajların ve kısıtlamaların farkında olarak en uygun davranış deneyini tercih etmelidir. Bu derlemede Alzheimer, epilepsi, migren, nöropatik ağrı, şizofreni, Parkinson, serebral iskemi ve travmatik beyin hasarı gibi deneysel modellerde en sık kullanılan öğrenme, hafıza, anksiyete, depresyon ve motor fonksiyon davranışsal deney protokollerinin detaylı olarak tanımlanması amaçlandı.
Anahtar kelimeler: Anksiyete; depresyon; hafıza; öğrenme; motor fonksiyon.

Corresponding Author
Sorumlu Yazar
Güven AKÇAY
guvenakcayibu@gmail.com

Received / Geliş Tarihi : 07.03.2024
Accepted / Kabul Tarihi : 07.05.2024
Available Online / Çevrimiçi Yayın Tarihi : 31.05.2024

INTRODUCTION

Experimental animals; human health protection, improvement, and complementary therapy prioritized in the discovery, and development of methods is a group of living organisms. A medicine or treatment method can be used on human beings through many trials and researches in order to become must pass. Animal models are the most preferred research method for the prevention and treatment of diseases in humans (1).

Behavioral experiments have been conducted since the classical conditioning research of Ivan Pavlov in 1904, and human behavior has been investigated through experiments on animals. The effectiveness of these models is tested by behavioral experiments such as cognitive, emotional, and motor functions such as learning, memory, locomotor activity, anxiety, and depression. The complex structure and signaling pathways of nervous systems make it difficult to understand the neurobiology and pathophysiology of diseases and also prevent the development of radical treatments for these diseases. Experimental studies play an important role in understanding the mechanisms of neurological diseases, preventing these diseases, and applying effective treatment methods. Research using animal models plays a vital role in elucidating the mechanisms of these diseases and developing effective treatment strategies (2). Rodents (e.g. mice, rats, gerbils) and non-rodent mammals (e.g. dogs, rabbits, cats, pigs, chimpanzees) are frequently used in the research of a treatment modality, the development of new treatment protocols, and the discovery of new drug molecules. In animal models, basic neuronal mechanisms of both normal and abnormal brain function are investigated. Each model has its own advantages and disadvantages. Therefore, the researcher should be aware of these advantages and limitations before choosing a particular model and should prefer the most appropriate model for his/her study (2).

In order to demonstrate the efficacy of a drug or treatment in animal experiments, the accuracy of the animal model must first be tested. In this review, the most commonly used learning, memory, anxiety, depression, and motor function behavioral experimental protocols in experimental models such as Alzheimer's, epilepsy, migraine, neuropathic pain, schizophrenia, Parkinson's, cerebral ischemia, and traumatic brain injury, etc. (3-8) were described in detail.

MOTOR FUNCTION BEHAVIOR EXPERIMENTS

Open Field Test

Open field test (OF) is a test generally used to assess locomotor activity (9). OF experiments are measured in a square experimental setup with a height of 40 cm and 80x80 cm in rat experiments, and in a square experimental setup with a height of 40 cm and 40x40 cm in mouse experiments (Figure 1). The areas are usually divided into 16 small squares equal to each other. At the beginning of the experiment, the animals are placed one by one in the center of this area and their movements are recorded by the camera for 5 minutes. Before each test, the OF setup must be thoroughly cleaned against the odor stimulus. The total distance (cm), velocity (cm/s), and the number of squares entered are measured as locomotor activity parameters (9).

Rotarod Test

The rotarod test is used to measure motor performance and coordination of animals (Figure 2). Animals are acclimatized to the rotarod for 3 consecutive days before the experiment. During this time, animals (for rats) are trained to walk on a rotating rod at a constant speed of 2 rpm for a maximum of 12 min. On the test day, after the animals are placed in their individual compartments in the rotarod device in the opposite direction of the rod (7.3 cm diameter) rotating at a uniform speed between 4 and 40 rpm for a period of 5 min, the time spent on the rod is automatically recorded (10). The recording time is set to 300 seconds and 3 consecutive measurements are made with rest periods of 5 minutes.

LEARNING AND MEMORY BEHAVIORAL EXPERIMENTS

Morris Water Maze

The Morris water maze test is highly preferred in learning and memory experiments. Especially working and reference memory are assessed. A circular water tank with a diameter of 150 cm and a height of 45 cm is used in the Morris Water Maze test setup. In the experimental room, visual clues are placed around the experimental setup. The height of the water should be 30 cm and the temperature should be 22±2 C^{o}. The circular tank is divided into 4 equal quadrants and a 10 cm diameter platform is placed 2 cm below the water surface in the escape quadrant. In the learning phase of the experiment, rats/mice are randomly released into the tank and allowed to swim for 90 seconds. Animals that find the platform within this time are allowed to stay on the platform for 30 seconds. Within 120 seconds, rats/mice that could not find the platform were slowly guided to the platform and allowed to stay on the platform for 30 seconds. For 5 days, 4 trials are performed daily at 30-minute intervals and the platform is removed at the end of the trials on the 6th day. During the experiment, whether learning occurred or not was evaluated with a 30-second probe trial in a water tank without a platform. Meanwhile, the arrival time of the mice to the platform, swimming paths, and swimming speeds of the mice were recorded by a video camera system (11).

Radial Arm Maze

The radial arm maze (RAM) is used to measure spatial learning and memory. The RAM consists of eight arms with a food zone at the end of the arm. In the room where the experimental setup is located, the RAM is surrounded by a large number of visual objects. Animals are habituated by exploring the maze for 5 minutes a day for 3 days. On the first day of habituation, animals are gradually allowed to access food from all arms. Following habituation, each trial is administered twice a day for 4 days. The 2nd, 3rd, 5th, and 7th arms have chocolate, while the other arms have no chocolate. The animal is placed in the center of the apparatus on each trial and working and reference memory are assessed (12). The maze was thoroughly cleaned with 70% ethanol and dried before each trial. With a video monitoring system in RAM, it is usually measured by three parameters; (i) the number of reference memory errors (RME, visits to unbaited arms), (ii) the number of working memory errors (WME, visits to previously visited arms in the same trial) and (iii) the accuracy index (number of first entries to baited arms/total

entries to all arms). Reference memory is associated with long-term memory for information that remains consistent across repeated trials (memory for the positions of unbaited arms), whereas working memory is associated with short-term memory, where the information to be recalled changes on each trial (memory for the positions of arms that have already been visited on each trial).

Novel Object Recognition Test

The novel object recognition test is especially used in attention and short-term memory studies and consists of three stages: habituation, training, and retention (Figure 3). In the habituation phase, animals are placed in the center of a 40 cm high, 80x80 cm setup and allowed to walk around for 5 minutes without any objects in the environment. In the training phase, animals are left in the center and allowed to examine two objects placed in the environment for 5 minutes. Between the phases, the apparatus should be cleaned with 70% ethanol to prevent the animals from moving according to the sense of smell. In the retention phase, one of the objects is replaced with a novel object, and the animals' behavior is recorded for 5 minutes. In this process, animals are expected to spend more time examining the novel object (13). In the novel object recognition test, the discrimination index and time spent on the novel object (s) values are analyzed. Discrimination Index = ((Time spent on the new object - Time spent on the old object) / Total time)*100.

Object Location Test

The object location test is especially used in short-term and spatial memory researches and consists of three stages: habituation, training, and retention (Figure 4). In the habituation phase, animals are placed in the center of a

Figure 1. Locomotor activity experimental phases

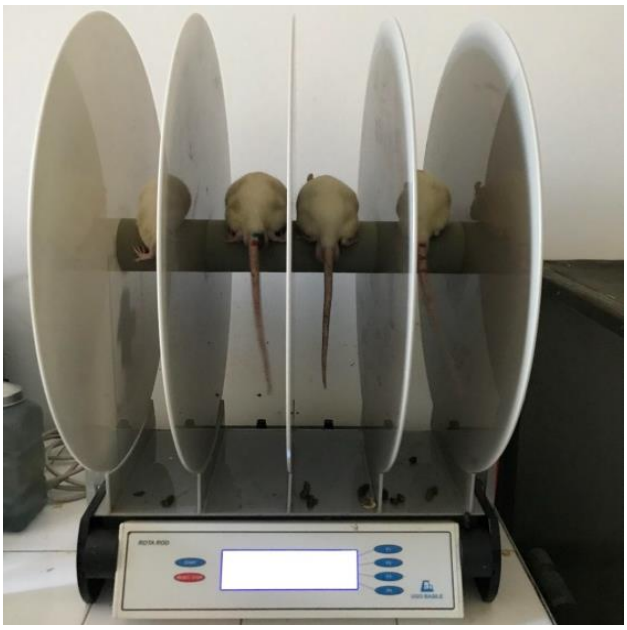

Figure 2. Rotarod system

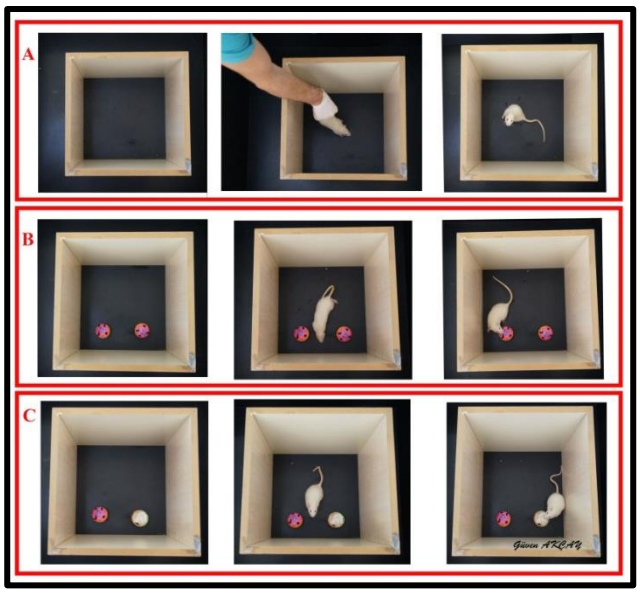

Figure 3. Experimental phases of novel object recognition test; a) habituation, b) training, and c) retention phases

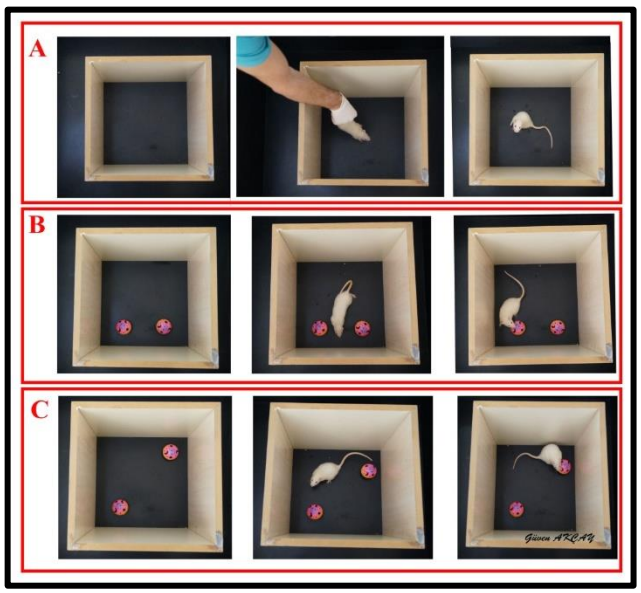

Figure 4. Experimental phases of the object localization test; a) habituation, b) training, and c) retention phases

40 cm high, 80x80 cm environment, and allowed to move around for 5 minutes without any objects in the environment. In the training phase, the animals are released from the center and allowed to examine two objects placed in the environment for 5 minutes. Between the stages, the maze setup is cleaned with 70% ethanol to prevent the animals from moving according to the sense of smell. In the recall phase, one of the objects is replaced and the behavior of the animals is recorded for 5 minutes. Animals are expected to spend more time examining the relocated object (14). In the object localization test, the discrimination index and the time spent on the displaced object (s) values are analyzed. Discrimination Index = ((Time spent on the displaced object - Time spent on the non-displaced object) / Total time)*100.

Y-maze Test

The Y-maze test is widely used to assess both the short-term memory and spatial memory of rats (9). The Y-maze test is a three-arm apparatus for animals, each arm is 50 cm long, 20 cm wall height, and 10 cm width, and the angle between the arms is 120° (Figure 5). The walls of the experiment room are equipped with visual clues. The arms of the maze are named as 'initial', 'other', and 'new' arms. In the first part of the experiment, rats are left at the end of the initial arm, and each animal is given 15 minutes to freely examine the other arms while the new arm is completely closed. At the end of 15 minutes, the animals are returned to the cages and the Y-labyrinth apparatus is cleaned with 70% ethanol to prevent them from moving according to the sense of smell during the experiment. One hour later to test short-term memory and 24 hours later to test long-term memory, a new arm is opened and the animals are returned to the initial arm of the maze and allowed to move freely in all three arms for 5 minutes and their behavior is recorded by a camera. To evaluate memory, the rate of entry into the new arm and the rate of time spent in the new arm are analyzed (9).

ANXIETY BEHAVIORAL EXPERIMENTS

Open Field Test

The open-field test is a test used to assess anxiety and some depression-like behaviors (Figure 6). The number of crossovers and the percentage of time spent in the outer/inner quadrant are used as depression parameters (3).

Elevated Plus Maze Test

It is a preferred test, especially in the determination of emotional behaviors and in the interpretation of long-term anxiety responses. Animals are housed in a plus-shaped enclosure with an arm width of 10 cm, an arm length of 45 cm, a wall height of 9 cm, and a floor height of 68 cm. Two arms are surrounded by walls and the other two arms are completely open. Each animal is released at the center where the arms overlap and its behavior is recorded for 5 minutes. The time spent in the open and closed arms and the number of entries into the open and closed arms recorded by the video camera are evaluated (15).

DEPRESSION BEHAVIORAL EXPERIMENTS

Forced Swim Test

The forced swim test is a test used in depression research, especially in testing short-term depression. In this model, animals are placed in a cylinder filled with water. After a certain period of time, the animals assume a posture of "immobility" in the water, a posture that reflects the depressed state of mind of animals whose body shape has lost hope (Figure 7). Symptoms of depression: Hopelessness, immobility, reduced escape behavior. Parameters measured: time spent in escape behavior, immobility, swimming, climbing, etc. Antidepressant effect: significant reduction in immobility (16).

Light-Dark Model

Animals are placed in a 60×60×60×45 cm 2-compartment set-up consisting of a light and a dark compartment. The chamber wall has an opening of 7×7 cm between the two compartments. The bright and novel environment causes

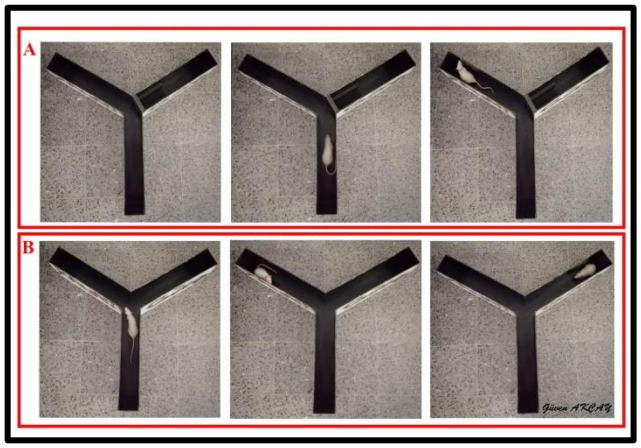

Figure 5. Y maze test experiment phases; a) training, and b) retention phases

Figure 6. Presentation of the path traveled by the experimental groups in the open field setup with lines and inner/outer quadrant transitions

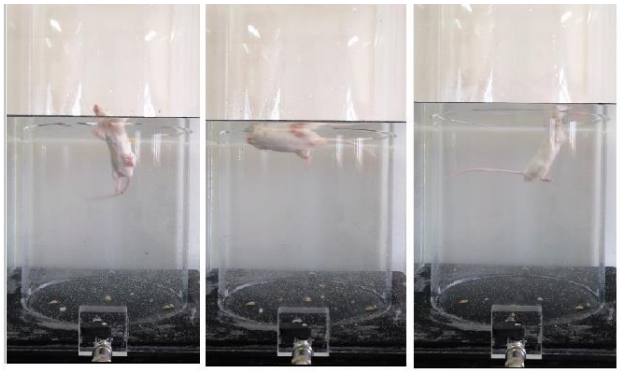

Figure 7. Forced swim test setup

photophobia for the animals and therefore the time spent in the dark area (movement) and the number of transitions is a sign of photophobic behavior. For 5 minutes, the time spent in both areas, the time spent moving in both areas and the number of transitions between the compartments are measured (17).

Catatonia Test

Catatonia is a psychomotor syndrome in which motor excitement, stereotypy, and stupor can be observed. Rats with genetic catatonia and pendulum-like movements in the anterior half of the body have physiological and behavioral changes similar to those observed in schizophrenia and depression in humans and can be considered as incomplete experimental models of these pathologies (18). Catatonia, a postural disorder, is defined as "freezing" in animals and is a simple and easily applicable method (10). Animals are slowly placed on a 25x35 cm vertical wire grid (5 mm spacing). The time the animals are completely immobilized on the grid is measured and recorded with a stopwatch (Figure 8). The severity of cataleptic behavior is assessed by measuring the longest period of immobility within a 2-minute observation period (10).

NEUROPATHIC PAIN BEHAVIORAL EXPERIMENTS

Hot Plate Test

The hot plate test assesses thermal hyperalgesia and its effects on the thermal nociceptive threshold (4,19). The surface of the hot plate apparatus was preheated and maintained at a constant temperature of 55±0.1 °C (Figure 9). Animals are placed into glass funnels on the heated surface and the time between the rat's placement and the first response (foot licking, jumping, or rapid raising of the paws) is recorded as the paw withdrawal latency. The cut-off time is set at 20 seconds to avoid tissue damage. The hind paw retraction time (sec) is measured for each animal (20).

Tail Flick Test

Thermal hyperalgesia is assessed by tail-flick test (21). The animal automatically raises its tail when it feels uncomfortable. Briefly, 2 cm of the distal tail is immersed in a water bath at 52.5±0.2 °C. The time for the animals to shake the tail is recorded as the tail shake latency; to avoid damage to the tail tissues, the cutting latency is set at 15 seconds (4,22).

Randall-Selitto Test

Mechanical hyperalgesia is measured with the Randall-Selitto test. The Randall-Selitto test involves applying evenly increasing mechanical pressure to the animal's paw (23). This pressure causes pain leading to an escape response. Animals are immobilized and grasped with one hand. Their hind paws are subjected to a linear increasing pressure until the paw retracts or vocalizations occur. The force (grams) with which the paw is withdrawn is recorded. 3-4 consecutive measurements are made at 5 min intervals and the retraction threshold for each animal is calculated by averaging the force at which the animal retracts the paw (Figure 10). The cutting force is determined as 250 g. The average of three consecutive tests with 1-minute inter-stimulus intervals is considered as the muscle pressure threshold (4,24).

Acetic Acid-Induced Writhing Test

Subjects are injected intraperitoneally with 0.3% acetic acid (10 ml/kg) to induce hyperalgesia. 5 minutes after the administration of acetic acid, the writhing (abdominal stretching/contraction) of the subjects is monitored for 5 minutes and the number of writhing is evaluated (25).

Figure 8. Catatonia test setup

Figure 9. Hot plate test setup

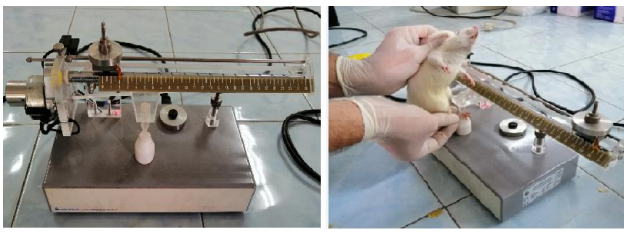

Figure 10. Randall-Selitto test setup

CONCLUSION

Behavioral animal experiments play an important role in understanding the biological basis of human behavior and disease. Studying with animals ensures high scientific quality, reliable and reproducible results. There are many different types of behavioral experiments. They all aim to test the accuracy of behavior in subjects, such as locomotor activity, learning, memory, depression, anxiety, and pain, as well as the effectiveness of the treatment.

Ethics Committee Approval: Since our study was a review, ethics committee approval was not required.

Conflict of Interest: None declared by the authors.

Financial Disclosure: None declared by the authors.

Acknowledgments: The author thanks to Dr. Dilara NEMUTLU SAMUR for her support in the experimental studies and the editing of the experimental photographs.

Author Contributions: Idea/Concept: GA; Design: GA; Data Collection/Processing: GA; Analysis/Interpretation: GA; Literature Review: GA; Drafting/Writing: GA; Critical Review: GA.

REFERENCES

1. Bovenkerk B, Kaldewaij F. The use of animal models in behavioural neuroscience research. Curr Top Behav Neurosci. 2015;19:17-46.
2. Agar A, Akcay G. Nörolojik hastaliklarin deneysel hayvan modelleri, vol 1. Ankara: Akademisyen Kitabevi; 2022. Turkish.
3. Akcay G, Derin N. The effects of tDCS on depression and anxiety disorders induced by sub-chronic stress. Turk Hij Den Biyol Derg. 2022;79(2):267-78.
4. Akcay G, Nemutlu Samur D, Derin N. Transcranial direct current stimulation alleviates nociceptive behavior in male rats with neuropathic pain by regulating oxidative stress and reducing neuroinflammation. J Neurosci Res. 2023;101(9):1457-70.
5. Akçay, G. Weight drop models in traumatic brain injury. Mid Blac Sea J Health Sci. 2023;9(2):375-84.
6. Akcay G. Therapeutic effects of transcranial direct current stimulation on ketamine-induced schizophrenia-like behaviors and oxidative stress. Med Science. 2023;12(1):63-9.
7. Akcay G, Tamerer S. Transcranial direct current stimulation ameliorates motor and cognitive functions by regulating neuronal excitotoxicity in experimental Parkinson's disease model. Cukurova Med J. 2023;48(3):919-28.
8. Akcay G, Baydemir R. Therapeutic effects of transcranial direct current stimulation on loss of motor function caused by experimental mild traumatic brain injury. Cukurova Med J. 2023;48(3):972-8.
9. Akçay G, Aslan M, Kipmen Korgun D, Çeker T, Akan E, Derin N. Effects of transcranial direct current stimulation on the glutamatergic pathway in the male rat hippocampus after experimental focal cerebral ischemia. J Neurosci Res. 2024;102(1):e25247.
10. Nemutlu Samur D, Akçay G, Yıldırım S, Özkan A, Çeker T, Derin N, et al., Vortioxetine ameliorates motor and cognitive impairments in the rotenone-induced Parkinson's disease via targeting TLR-2 mediated neuroinflammation. Neuropharmacology, 2022;208:108977.
11. Danışman B, Akçay G, Gökçek-Saraç Ç, Kantar D, Aslan M, Derin N. The role of acetylcholine on the effects of different doses of sulfite in learning and memory. Neurochem Res. 2022;47(11):3331-43.
12. Özkan A, Bülbül M, Derin N, Sinen O, Akçay G, Parlak H, et al. Neuropeptide-S affects cognitive impairment and depression-like behavior on MPTP induced experimental mouse model of Parkinson's disease. Turk J Med Sci. 2021;51(6):3126-35.
13. Johnston IN, Tan M, Cao J, Matsos A, Forrest DRL, Si E, et al. Ibudilast reduces oxaliplatin-induced tactile allodynia and cognitive impairments in rats. Behav Brain Res. 2017;334:109-18.
14. Wagner BA, Braddick VC, Batson CG, Cullen BH, Miller LE, Spritzer MD. Effects of testosterone dose on spatial memory among castrated adult male rats. Psychoneuroendocrinology. 2018;89:120-30.
15. Akcay G, Bahadir A, Tatar Y, Nuri Atalar M, Babur C, Taylan Ozkan A. Investigation of the effects of Toxoplasma gondii on behavioral and molecular mechanism in bradyzoite stage. Brain Res. 2024;1828:148762.
16. Zhao Y, Li H, Fang F, Qin T, Xiao W, Wang Z, Ma S. Geniposide improves repeated restraint stress-induced depression-like behavior in mice by ameliorating neuronal apoptosis via regulating GLP-1R/AKT signaling pathway. Neurosci Lett. 2018;676:19-26.
17. Kawashita E, Kanno Y, Ikeda K, Kuretake H, Matsuo O, Matsuno H. Altered behavior in mice with deletion of the alpha2-antiplasmin gene. PLoS One. 2014;9(5):e97947.
18. Plekanchuk VS, Prokudina OI, Ryazanova MA. Social behavior and spatial orientation in rat strains with genetic predisposition to catatonia (GC) and stereotypes (PM). Vavilovskii Zhurnal Genet Selektsii. 2022;26(3):281-9.
19. Cioato SG, Medeiros LF, Marques Filho PR, Vercelino R, de Souza A, Scarabelot VL, et al. Long-lasting effect of transcranial direct current stimulation in the reversal of hyperalgesia and cytokine alterations induced by the neuropathic pain model. Brain Stimul. 2016;9(2):209-17.
20. Santos DS, Lopes BC, Medeiros LF, Assumpção JAF, de Souza A, Salvi AA, et al. Transcranial direct current stimulation (tDCS) induces analgesia in rats with neuropathic pain and alcohol abstinence. Neurochem Res. 2020;45(11):2653-63.
21. Hacısüleyman L, Saraç B, Joha Z. Analgesic effects of vilazodone, indatraline, and talsupram in a rat model of neuropathic pain. Turk J Pharm Sci. 2022;19(3):336-42.
22. Arslan R, Aydin S, Nemutlu Samur D, Bektas N. The possible mechanisms of protocatechuic acid-induced central analgesia. Saudi Pharm J. 2018;26(4):541-5.
23. Zhang Y, Ma S, Ke X, Yi Y, Yu H, Yu D, Li Q, Shang Y, Lu Y, Pei L. The mechanism of Annexin A1 to modulate TRPV1 and nociception in dorsal root ganglion neurons. Cell Biosci. 2021;11(1):167.

24. De la Luz-Cuellar YE, Rodríguez-Palma EJ, Franco-Enzástiga Ú, Déciga-Campos M, Mercado F, Granados-Soto V. Spinal dopaminergic D1 and D5 receptors contribute to reserpine-induced fibromyalgia-like pain in rats. Brain Res. 2023;1799:148167.
25. Girotra P, Singh SK. A comparative study of orally delivered PBCA and ApoE coupled BSA nanoparticles for brain targeting of sumatriptan succinate in therapeutic management of migraine. Pharm Res. 2016;33(7):1682-95.