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Review Article

Study of Mouse Behavior in an Animal Facility

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Abstract

Behavioral studies in laboratory mice represent a cornerstone of preclinical biomedical research, providing valuable experimental models for investigating the mechanisms underlying human neurological and psychiatric disorders. The large number of publications in this field highlights the importance of mouse behavioral paradigms for understanding disease pathophysiology and evaluating the efficacy of potential therapeutic interventions before clinical application. Several validated models have been developed to reproduce distinct behavioral phenotypes associated with psychiatric disorders. Depression is commonly investigated using paradigms such as bilateral olfactory bulbectomy, learned helplessness, chronic mild stress, and chronic social defeat stress. Anxiety-like behavior is frequently assessed through tests including the elevated plus maze, elevated zero maze, elevated T-maze, and light–dark box, which exploit rodents' natural conflict between exploration and avoidance of potentially threatening environments. Aggression is most often evaluated using the resident–intruder paradigm, a well-established model for studying territorial behavior, social hierarchy, and stress-induced aggressive responses. Behavioral paradigms are equally important for studying neurodevelopmental disorders. In mouse models of Autism Spectrum Disorder (ASD), the three-chamber social approach test is widely employed to assess deficits in sociability and preference for social novelty, two core characteristics of the disorder. Additional assays examining reciprocal social interaction and repetitive behaviors further contribute to the characterization of ASD-related phenotypes. Neurodegenerative diseases also rely heavily on behavioral evaluation. In Alzheimer's disease models, cognitive performance, memory, exploratory activity, and social behavior are commonly assessed using tasks such as the Y-maze, open field, and social interaction tests. In contrast, Parkinson's disease models primarily focus on motor impairment through spontaneous locomotor activity, the cylinder test, and the rotarod, while cognitive tasks are increasingly incorporated to evaluate non-motor symptoms. Overall, mouse behavioral models provide reliable and translational tools for reproducing key aspects of human disease. Their continued refinement has significantly advanced our understanding of disease mechanisms and remains essential for the development and preclinical validation of novel therapeutic strategies.

Keywords: Mice, Behavior, Psychiatric disorders, Behavioral tests

Introduction

Behavioral research in mice represents a broad and multidisciplinary area of preclinical investigation, encompassing numerous experimental paradigms for modeling human diseases. More than 138,000 studies indexed in scientific databases have explored different aspects of mouse behavior, highlighting the importance of these models in biomedical research. Depression and anxiety are among the most extensively investigated conditions, followed by neurodevelopmental disorders such as autism

spectrum disorder. Behavioral assessments also play a central role in neurodegenerative disease research, particularly in experimental models of Alzheimer's and Parkinson's diseases, where they are widely used to evaluate cognitive and motor impairments.

Major Depression

Depression is a major public health concern worldwide, affecting millions of people and imposing a substantial burden on individuals and healthcare systems. In the United States, approximately 6.7% of the population will experience depression at some point during their lifetime, corresponding to nearly 35 million adults who are expected to undergo at least one major depressive episode [1]. Beyond its high prevalence, depression is one of the leading causes of disability, generating an estimated economic burden of $83.1 billion in 2000 [2]. Despite the availability of several antidepressant therapies, only about half of patients achieve a satisfactory clinical response [3]. This limited effectiveness may be explained by the fact that depression is currently diagnosed exclusively on the basis of behavioral symptoms, while existing pharmacological treatments are not designed to target the underlying biological mechanisms of the disorder [4,5].

Reproducing depression-like states in animal models remains a significant challenge because of the complex and multifaceted nature of the disorder, which involves subjective psychological manifestations, diverse physiological alterations, and the absence of reliable objective biomarkers [6]. The usefulness of an experimental model is commonly assessed on three key criteria: its ability to reproduce features comparable to those observed in human depression, the feasibility of objectively quantifying the resulting behavioral alterations, and the responsiveness of these alterations to clinically effective antidepressant treatments [7]. Among the available experimental approaches, bilateral olfactory bulbectomy (OBX) has been extensively employed for more than four decades in both rats and mice as a preclinical model for evaluating antidepressant efficacy [8]. Removal of the olfactory bulbs disrupts the cortical–hippocampal–amygdala network, leading to profound neurobehavioral alterations, including changes in locomotor activity, feeding behavior, and avoidance responses [9]. In addition, OBX animals exhibit impairments in cognition, diminished sexual behavior, reduced social interaction, and decreased exploratory activity in unfamiliar environments, alterations that have been associated with cortical neuronal degeneration [10]. Although the translational relevance of the OBX model to human depression remains under debate, clinical evidence supports an association between olfactory dysfunction and depressive disorders. Patients with major depression frequently present reduced olfactory sensitivity, and the severity of depressive symptoms has been shown to correlate inversely with olfactory bulb volume [11]. Interestingly, experimental models based on social deprivation and alterations in maternal care produce behavioral and neurochemical outcomes that vary according to the developmental stage of the offspring [12]. These findings emphasize the need for further investigations aimed at identifying the causal mechanisms involved in the emergence of depressive-like behaviors during critical developmental periods, thereby facilitating the translation of experimental evidence to human depressive disorders. Similar to what is observed in humans, only a proportion of male rats exposed to uncontrollable stress develop susceptibility to Learned Helplessness (LH), which is characterized by enhanced depressive-like responses [13,14]. In contrast, female rodents do not exhibit the classical LH phenotype, underscoring the importance of considering sex differences when interpreting data from animal models and when developing novel antidepressant therapies [15]. Repeated restraint stress in adult rodents represents another widely used experimental paradigm. Animals subjected to this procedure display a depression-like phenotype characterized by reduced preference for sucrose, impaired exploratory behavior associated with anxiety, and increased immobility in the Forced Swim Test (FST) [16]. Notably, several of these alterations are reversed only after prolonged antidepressant treatment, rather than after acute drug administration. Another important experimental approach is the Chronic Mild Stress (CMS) model, which is based on the hypothesis that prolonged exposure to mild, unpredictable stressors resembling everyday human stress can trigger depressive-like states in susceptible individuals [17]. CMS produces a broad spectrum of behavioral disturbances, including reduced sucrose consumption, decreased motivation for rewarding stimuli, diminished sexual behavior, increased aggression, anxiety-related responses, and disturbances in sleep patterns [18]. As observed in other validated depression models, these behavioral impairments are generally responsive to chronic, but not acute, treatment with conventional antidepressants. In addition, Chronic Social Defeat Stress (CSDS) has emerged as a robust rodent model for studying stress-induced depression. Repeated exposure to social defeat produces a phenotype characterized by anhedonia and social avoidance, and these behavioral abnormalities can be attenuated or reversed following chronic antidepressant treatment [19].

Anxiety

Anxiety and fear are evolutionarily conserved emotional responses that play essential roles in promoting survival and adaptation. Although fear is typically elicited by an identifiable and immediate threat, anxiety is generally characterized by anticipation of uncertain or poorly defined danger [20]. While adaptive anxiety is a normal component of defensive behavior, pathological anxiety is excessive, persistent, and disruptive, significantly compromising daily functioning and imposing a substantial burden on healthcare systems worldwide [21,22]. Experimental studies of anxiety frequently rely on behavioral paradigms that exploit rodents' innate conflict between the drive to explore novel environments and the avoidance of exposed areas. Among these, the Elevated Plus Maze (EPM) is one of the most extensively validated and widely used animal models for assessing anxiety-like behavior. Factor analysis of behavioral parameters recorded during the standard 5-minute test has demonstrated that the number of entries into the

enclosed arms provides a reliable measure of general locomotor activity, whereas the proportion of entries into, and time spent in, the open arms represents the principal indicator of anxiety-like behavior [23]. Consistent with the predictive validity of the model, anxiolytic compounds such as diazepam, administered at doses that do not impair motor function, selectively increase exploration of the open arms without altering activity in the enclosed arms [24]. The Elevated Zero Maze (EZM) was subsequently developed as an alternative to the EPM, preserving the same approach–avoidance conflict while eliminating the central platform, whose interpretation can be ambiguous in the EPM [25]. In addition to incorporating conventional behavioral measures, the EZM allows the assessment of several ethologically relevant parameters that improve the evaluation of pharmacological interventions. As observed in the EPM, anxiety-like behavior in the EZM is primarily quantified by the percentage of entries into and the proportion of time spent in the open sections of the apparatus during the test session [26]. The elevated T-maze (ETM), an adaptation of the elevated plus maze, was designed to evaluate distinct defensive behaviors within the same animal. The apparatus consists of one enclosed arm positioned perpendicular to two opposing open arms of identical dimensions. This paradigm simultaneously assesses inhibitory avoidance, a conditioned response associated with generalized anxiety, and escape behavior from the open arms, an unconditioned response considered analogous to panic-related behavior [27]. Another well-established paradigm for assessing anxiety-like behavior is the light–dark box test, which predates the development of the elevated plus maze. During a standard 5-minute session, rodents are allowed to move freely between two connected compartments that differ in illumination: a dark, protected area and a brightly lit, exposed area. Because rodents naturally avoid illuminated environments while maintaining a strong motivation to explore novel surroundings, this test exploits the conflict between these opposing drives [28]. The anxiolytic validity of the model has been demonstrated by the effects of benzodiazepines, which increase both the time spent in the illuminated compartment and the frequency of transitions between the two chambers without markedly affecting locomotor activity [28]. The hole-board test provides an additional measure of anxiety-related behavior by evaluating exploratory head-dipping. In this paradigm, rodents are placed in an arena containing evenly distributed holes in the floor, through which they spontaneously insert their heads to investigate the environment. The frequency of head-dipping is widely accepted as an indicator of exploratory motivation and is generally considered to be inversely related to anxiety levels, with reduced exploratory behavior reflecting increased anxiety-like states [29]. Social behavior can also be used to assess anxiety through the social interaction test, in which two unfamiliar rodents are allowed to interact freely within an open arena while the duration of their social behaviors is recorded. Since the behavior of each animal directly influences that of its partner, the pair is treated as a single experimental unit during data analysis [30]. A complementary approach is the hyponeophagia paradigm, which evaluates the conflict between hunger and fear of novelty. Following approximately 24 hours of food deprivation, animals are placed in an unfamiliar transparent chamber containing a food pellet positioned on a centrally located platform under bright illumination. The latency to approach the food and begin eating serves as the primary behavioral endpoint, with longer latencies indicating higher levels of anxiety-like behavior [31]. Evidence from both preclinical and clinical studies indicates that exposure to stressful events across the lifespan plays a fundamental role in the development of psychiatric disorders, including anxiety disorders, mood disorders, and schizophrenia [32]. In particular, chronic stress activates the Hypothalamic–Pituitary–Adrenal (HPA) axis, resulting in sustained alterations in endocrine function, including changes in glucocorticoid secretion and other stress-related hormonal mediators. These neuroendocrine adaptations are accompanied by behavioral abnormalities that can be detected in experimental models of anxiety. Likewise, adverse experiences during early development, such as maternal separation or neonatal isolation, induce persistent changes in brain function and behavior, increasing vulnerability to psychiatric disorders and producing long-term consequences for emotional regulation and overall quality of life.

The neonatal maternal separation paradigm is a widely used experimental model for investigating the long-term consequences of early-life stress. In this protocol, beginning on postnatal day 2, each litter is removed from the home cage and transferred to a separate cage for approximately 1 hour, typically between 9:00 a.m. and 12:00 p.m., in a room isolated from the main animal facility. To minimize interference from auditory cues, white noise is continuously played to mask vocalizations from other litters. At the end of each separation session, the pups are returned to their dam in the home cage. This procedure is repeated daily for eight consecutive days. The neonatal separation model has been extensively employed to investigate how adverse experiences during early development increase susceptibility to addiction and promote anxiety-like phenotypes that become evident during adulthood in behavioral paradigms such as contextual fear conditioning, the Elevated Plus Maze (EPM), and the social interaction test [33]. Physical restraint and immobilization paradigms are among the most frequently used methods for inducing experimental stress in laboratory rodents, as they reliably produce behavioral, physiological, and biochemical alterations associated with stress responses. In restraint stress protocols, animals are typically confined within ventilated cylindrical or semi-cylindrical restrainers for periods ranging from 120 to 180 minutes, thereby restricting movement while avoiding direct physical injury [34]. Immobilization stress represents a more severe form of restraint, in which both the forelimbs and hindlimbs are gently secured with adhesive tape and head movement is further limited by a metal loop positioned around the neck. Depending on the experimental design, immobilization may be applied as either an acute challenge or repeatedly over periods of 7 to 21 days to model chronic stress. Because these procedures expose animals to uncontrollable physical and psychological stress with minimal habituation, they are considered highly effective for

eliciting sustained stress responses. Following repeated restraint or immobilization, rodents consistently display increased anxiety-like behavior, evidenced by reduced exploration of open arms in the elevated plus maze and similar alterations across other validated anxiety assays [35].

Aggressive Behavior

Aggressive behavior in group-housed male mice has been investigated using a variety of experimental paradigms designed to assess different aspects of social interaction and dominance. One of the most commonly employed approaches involves the direct observation of undisturbed animals within their home cages, allowing spontaneous aggressive behaviors to be recorded under relatively natural social conditions [36]. In addition, several standardized behavioral assays have been developed to quantify aggression and establish social hierarchy, including the resident–intruder test [37], the social defeat paradigm [19], the novel arena social encounter test [38], and the tube test [39]. These experimental models primarily evaluate territorial aggression by exposing animals to situations that evoke social conflict, emotional stress, and frustration, with aggressive behavior serving as the principal behavioral outcome [36]. Consequently, they have become valuable tools for investigating the neurobiological mechanisms underlying aggression, social defeat, impairments in social interaction, and mood-related psychiatric disorders in rodent models [40]. Unlike home cage observations, these paradigms generally require animals to be removed from their familiar social environment and introduced into a novel testing arena, where they encounter unfamiliar conspecifics under controlled experimental conditions designed to provoke specific social responses. In addition to direct behavioral assessment, aggression can also be evaluated indirectly through the systematic examination of physical injuries. The occurrence and severity of wounds are typically monitored throughout the experimental period and may also be quantified following euthanasia, providing an objective indicator of aggressive interactions among group-housed animals [36].

Experimental investigation of aggressive behavior has relied extensively on paradigms based on the resident–intruder model, one of the most widely validated approaches for studying social aggression in rodents. In this paradigm, an unfamiliar intruder is introduced into the territory of a resident animal, resulting in direct physical and social confrontation. The procedure is characterized by brief episodes of close interaction and aggressive attacks interspersed with prolonged periods during which the intruder remains in sensory contact with, but physically separated from, the resident. Unlike humans, whose cognitive processes allow psychological stress to persist even after removal of the stressor, rodents appear to require continuous sensory exposure for the maintenance of stress-related responses [37]. Findings obtained with this model indicate that individual differences in submissive behavior and stress susceptibility may be linked to enhanced cognitive performance. These observations suggest that cognitive abilities associated with social subordination may represent adaptive traits that exist prior to exposure to social hierarchies. From an evolutionary perspective, subordinate individuals may have developed compensatory behavioral strategies that improve survival, implying that, in the absence of social competition, naturally submissive animals could display superior performance in learning and other cognitive tasks [41]. Another widely used experimental approach is prolonged social isolation, which serves as a model for examining the behavioral and neurobiological consequences of chronic social deprivation. Male mice housed individually for periods exceeding four weeks consistently develop heightened aggressive behavior accompanied by profound neurochemical alterations. These include reduced sensitivity to drugs acting on GABA(A) receptors and decreased brain concentrations of the neurosteroid allopregnanolone (3α,5α-tetrahydroprogesterone; 3α,5α-THP), a potent positive allosteric modulator of GABA(A) receptor function. Reduced allopregnanolone levels have been associated with diminished expression of type I 5α-reductase at both the mRNA and protein levels, suggesting impaired neurosteroid biosynthesis. Interestingly, systemic administration of fluoxetine or its active metabolite norfluoxetine restores cerebral allopregnanolone concentrations and normalizes GABAergic responsiveness through stereospecific mechanisms [42]. Elucidating the biological basis of pathological aggression, whether driven by genetic predisposition or environmental influences, requires a detailed understanding of the neural circuits responsible for the regulation of normal aggressive behavior. Functional studies have identified neurons within the ventrolateral subdivision of the Ventromedial Hypothalamic Nucleus (VMHvl) as both necessary and sufficient for the expression of male–male aggression. This hypothalamic region, traditionally recognized for its role in reproductive behaviors, contains neuronal populations that partially overlap with those activated during male–female mating, with approximately one-fifth of the neurons participating in both behavioral responses [43]. These findings highlight the close neurobiological relationship between aggression and reproductive behaviors. More recently, increasing evidence has implicated the microbiota–gut–brain axis as an important modulator of aggressive behavior. Experimental studies using mice, hamsters, dogs, and Drosophila have demonstrated that alterations in gut microbial composition can influence aggression across multiple species. For example, disruption of the maternal intestinal microbiota during pregnancy increases aggressive behavior in the offspring, whereas manipulation of the gut microbiome during early postnatal development in germ-free mice suppresses aggressive responses. Together, these findings emphasize the critical contribution of early-life microbial colonization to the maturation of neural circuits involved in social behavior and suggest that interventions targeting the gut microbiota during development may represent a promising strategy for modulating aggression-related disorders [44].

Autism Spectrum Disorders and Attention-Deficit/Hyperactivity Disorder

Autism Spectrum Disorder (ASD) and attention-deficit/

hyperactivity disorder (ADHD) are common neurodevelopmental disorders that exhibit considerable clinical and genetic overlap, making differential diagnosis particularly challenging. Both conditions are characterized by impairments in attention, executive function, and social behavior, although they differ in their epidemiology, underlying pathophysiology, diagnostic criteria, and therapeutic approaches. Despite these distinctions, comorbidity between ASD and ADHD is frequently observed, highlighting the existence of shared biological mechanisms. Rodent models have been instrumental in investigating behavioral traits associated with ASD, particularly deficits in social interaction and the presence of repetitive behaviors. One of the most widely used paradigms is the three-chamber social approach test, which evaluates both sociability and preference for social novelty. The apparatus, typically constructed from transparent Plexiglas, consists of three interconnected compartments of similar dimensions. During the initial habituation phase, the test animal is confined to the central chamber to acclimate to the environment. Subsequently, an unfamiliar conspecific enclosed within a wire or metal cage is introduced into one of the side chambers, while an identical empty enclosure is positioned in the opposite chamber. Once the barriers separating the compartments are removed, the experimental mouse is allowed to explore the entire apparatus freely. The amount of time spent in proximity to the unfamiliar animal, as well as the duration of direct investigative behavior toward the enclosure, are used as indicators of sociability. In the final phase, a second unfamiliar mouse is placed inside the previously empty enclosure, allowing assessment of social novelty preference by comparing the interaction directed toward the novel animal with that directed toward the familiar conspecific. Reduced exploration of the unfamiliar mice or diminished preference for social novelty is considered indicative of ASD-like social deficits [45–47]. Social behavior can also be assessed using the reciprocal social interaction test. After acclimation to the experimental room and subsequently to a clean cage containing fresh bedding, an unfamiliar mouse is introduced into the enclosure, and both animals are permitted to interact freely. Throughout the observation period, multiple social behaviors—including sniffing, following, grooming, mounting, huddling, and wrestling—are quantified to provide a comprehensive assessment of social engagement. Rodents displaying ASD-like phenotypes consistently exhibit reductions in the frequency and duration of these social interactions [48]. Repetitive and restricted patterns of behavior, another hallmark of ASD, are commonly evaluated using the repetitive novel object test. Following habituation, animals are individually exposed to a testing arena containing several unfamiliar objects distributed throughout the cage. During the observation period, investigators record exploratory behaviors directed toward the objects, including repeated contacts and object burying. Quantitative measures such as the frequency of interaction with each object, the number of repetitive contacts across multiple objects, and the occurrence of burying behavior provide indices of behavioral stereotypy and repetitive activity, with increased repetitive responses reflecting ASD-like characteristics [49,50]. Animal models have also contributed substantially to the understanding of ADHD-related behavioral abnormalities. Experimental evidence indicates that prenatal exposure to maternal stress, particularly restraint stress during gestation, produces long-lasting behavioral alterations in the offspring that resemble core symptoms of ADHD. Adult mice exposed to prenatal stress display persistent hyperactivity, including increased voluntary wheel-running activity that remains elevated even after repeated habituation sessions. Pharmacological studies further demonstrate that dopamine receptor antagonists attenuate this hyperactive phenotype, supporting the involvement of dopaminergic signaling pathways. Consistent with these experimental findings, epidemiological studies have suggested an association between maternal stress during pregnancy and an increased risk of ADHD in children, although the precise neurobiological mechanisms linking prenatal stress to the disorder remain to be fully elucidated [51,52].

Alzheimer's Diseases

Alzheimer's Disease (AD) is the leading cause of dementia in older adults and is characterized by a progressive decline in memory and cognitive performance, accompanied by a broad spectrum of behavioral and neuropsychiatric disturbances [53]. Despite extensive research, the mechanisms underlying AD remain incompletely understood. Current evidence indicates that disease development results from the interaction of multiple factors, including genetic susceptibility, environmental influences, lifestyle, metabolic dysfunction, and traumatic brain injury [54]. Among these risk factors, advancing age is considered the strongest predictor of disease onset [55]. The global increase in life expectancy, driven by improvements in healthcare and living conditions, has contributed substantially to the growing prevalence of AD, making it one of the most significant public health challenges associated with population aging. Experimental mouse models have been widely employed to investigate the cognitive and behavioral impairments associated with AD. Spatial working memory is commonly evaluated using the Y-maze spontaneous exploration test. This paradigm is typically conducted in two sessions separated by an interval of approximately two hours. During the training phase, access to one of the three arms is prevented, allowing animals to explore only the remaining two arms. In the subsequent test phase, the previously inaccessible arm is opened, enabling unrestricted exploration of the entire maze. Memory performance is assessed by quantifying the preference for the novel arm, including the percentage of time spent in this arm and the number of entries, while locomotor activity is simultaneously evaluated through measurements of total distance traveled and average movement speed [56]. Exploratory behavior and anxiety-like responses are frequently examined using the open field test. In this assay, each mouse is placed in the center of a square arena and allowed to explore the environment freely for a predetermined period, generally 10 minutes. Because rodents naturally avoid open and brightly illuminated spaces, the amount of time spent in the central zone and the frequency of entries

into this area serve as indicators of anxiety-related behavior. In parallel, locomotor activity is quantified by recording parameters such as total distance traveled and mean velocity, providing complementary information that helps distinguish cognitive or emotional alterations from potential motor impairments [57].

Parkinson's Disease

Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder worldwide and is primarily characterized by the progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc), resulting in a marked depletion of dopamine within the striatum [58,59]. Another neuropathological hallmark of the disease is the abnormal aggregation of α-synuclein, which contributes to neuronal dysfunction and degeneration [60]. Although PD is classically recognized as a movement disorder, it encompasses a broad spectrum of both motor and non-motor manifestations that progressively impair patients' functional capacity and quality of life. The cardinal motor features of PD include bradykinesia, akinesia, muscular rigidity, resting tremor, and postural instability, all of which arise from dysfunction of the nigrostriatal dopaminergic pathway [61,62]. In addition to these motor impairments, patients frequently develop non-motor symptoms, including deficits in attention, recognition, executive function, and working memory, often accompanied by anxiety and depression. Importantly, these cognitive and psychiatric alterations may precede the onset of overt motor dysfunction, highlighting their relevance as early clinical manifestations of the disease [60,61]. Rodent models have become indispensable for investigating the behavioral consequences of dopaminergic degeneration and for evaluating potential therapeutic interventions. General locomotor activity is commonly assessed through spontaneous movement tests, which quantify parameters such as ambulation, exploratory behavior, rearing frequency, movement latency, and overall motor performance [63,64]. These behavioral assays have been extensively applied in both toxin-induced and genetically engineered models of PD to characterize disease progression and treatment efficacy. Forelimb motor asymmetry is frequently evaluated using the cylinder test, one of the most sensitive behavioral paradigms for detecting unilateral motor deficits. In this assay, rodents are placed individually inside a transparent vertical cylinder that allows unrestricted rearing behavior while permitting observation from multiple angles, often with the aid of a mirror positioned beneath the apparatus [65]. Motor performance is determined by quantifying forelimb contacts with the cylinder wall during spontaneous rearing episodes. Depending on the experimental design, analyses may include the total number of wall contacts or distinguish between the use of the ipsilateral and contralateral forelimbs relative to the lesioned hemisphere, thereby providing a sensitive measure of motor asymmetry and limb preference [65]. Another widely employed behavioral assessment is the pole test, which is particularly useful for detecting impairments in motor coordination and bradykinesia. During this procedure, the animal is positioned facing upward at the top of a vertically oriented wooden pole and is required to rotate its body before descending toward its home cage or a designated platform [66]. Performance is evaluated by measuring both the time required to orient downward and the total descent time, parameters that reliably reflect deficits in motor initiation, balance, and coordination associated with Parkinsonian phenotypes [66]. The rotarod test is one of the most extensively validated and widely employed behavioral assays for evaluating motor deficits in experimental models of Parkinson's Disease (PD). Originally introduced by Dunham and Miya in 1957, this paradigm remains a standard method for assessing motor coordination, balance, and motor learning in rodents [67]. As Parkinsonian pathology progresses, performance on the rotarod gradually deteriorates. Animals in the early stages of the disease exhibit only subtle reductions in the time they are able to remain on the rotating rod, whereas intermediate stages are characterized by increasingly impaired balance and shorter retention times. In advanced stages, rodents display pronounced motor dysfunction, with frequent falls and a substantial reduction in latency to fall, closely resembling the progressive motor decline observed in patients with advanced PD [67]. The rotarod apparatus consists of a horizontally positioned cylindrical rod, typically measuring 3–7 cm in diameter, which rotates at either a constant or an accelerating speed depending on the experimental protocol [68]. During testing, the animal is placed on the rotating rod and must continuously adjust its posture to maintain balance while avoiding a fall onto a platform positioned approximately 30 cm below. The primary outcome measure is the latency to fall, which provides a quantitative index of motor coordination, balance, and muscular endurance [69]. Owing to its simplicity, reproducibility, and minimal training requirements, the rotarod has become one of the most commonly used behavioral tests for evaluating motor impairment and therapeutic efficacy in rodent models of PD [69-71]. Beyond its characteristic motor manifestations, Parkinson's disease is increasingly recognized as a disorder accompanied by significant cognitive dysfunction. Deficits in attention, executive function, working memory, visual processing, and object recognition are frequently observed, often emerging during the early phases of the disease and contributing substantially to reduced quality of life [72–77]. Consequently, the assessment of cognitive performance has become an important component of preclinical studies, and numerous animal models of PD reproduce impairments in learning, memory, and attentional processes similar to those described in affected patients. Several behavioral paradigms have been developed to evaluate these cognitive alterations, among which the Morris water maze, the novel object recognition test, and the Y-maze are the most widely adopted. The Morris water maze is considered one of the principal methods for assessing hippocampus-dependent spatial learning and memory. In this task, rodents are required to locate a submerged escape platform hidden beneath the surface of an opaque water-filled circular pool by relying on distal visual cues distributed around the testing environment [78]. Cognitive performance is quantified using parameters such as escape latency,

swimming path length, and time required to locate the hidden platform. Progressive increases in escape latency or less efficient search strategies are interpreted as evidence of impaired spatial learning and memory. In experimental models of PD, animals often exhibit mild but measurable delays in locating the platform during the early stages of disease progression, reflecting the onset of cognitive dysfunction before the appearance of severe neurological impairment [78].

Ethical Considerations

There is no need for approval in ethics committees.

Declaration of Conflicting Interest

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References

1. Kessler RC, Chiu WT, Demler O, Merikangas KR, Walters EE (2005) Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. Arch Gen Psychiatry 62(6): 617-627.
2. Greenberg PE, Kessler RC, Birnbaum HG, Leong SA, Lowe SW, et al. (2003) The economic burden of depression in the United States: how did it change between 1990 and 2000? J Clin Psychiatry 64 (12): 1465-1475.
3. Brent DA, Kolko DJ, Birmaher B, Baugher M, Bridge J, et al. (1998) Predictors of treatment efficacy in a clinical trial of three psychosocial treatments for adolescent depression. J Am Acad Child Adolesc Psychiatry 37(9): 906-914.
4. Rush AJ, Trivedi MH, Wisniewski SR, Nierenberg AA, Stewart JW, et al. (2006) Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. Am J Psychiatry 163(11): 1905-1917.
5. Berton O, Nestler EJ (2006) New approaches to antidepressant drug discovery: beyond monoamines. Nat Rev Neurosci 7(2): 137-151.
6. Nestler EJ, Hyman SE (2010) Animal models of neuropsychiatric disorders. Nat Neurosci 13(10): 1161-1169.
7. McKinney Jr WT, Bunney Jr WE (1969) Animal model of depression. I. Review of evidence: implications for research. Arch Gen Psychiatry 21(2): 240-248.
8. Han F, Nakano T, Yamamoto Y, Shioda N, Lu YM, et al. (2009) Improvement of depressive behaviors by nefiracetam is associated with activation of CaM kinases in olfactory bulbectomized mice. Brain Res 1265: 205-214.
9. Song C, Leonard BE (2005) The olfactory bulbectomised rat as a model of depression. Neurosci Biobehav Rev 29: 627-647.
10. Wang D, Noda Y, Tsunekawa H, Zhou Y, Miyazaki M, et al. (2007) Behavioural and neurochemical features of olfactory bulbectomized rats resembling depression with comorbid anxiety. Behav Brain Res 178(2): 262-273.
11. Atanasova B, Graux J, El Hage W, Hommet C, Camus V, et al. (2008) Olfaction: a potential cognitive marker of psychiatric disorders. Neurosci Biobehav Rev 32(7): 1315-1325.
12. Weinstock M (2008) The long-term behavioural consequences of prenatal stress. Neurosci Biobehav Rev 32(6): 1073-1086.
13. Pryce CR, Azzinnari D, Spinelli S, Seifritz E, Tegethoff M, et al. (2011) Helplessness: a systematic translational review of theory and evidence for its relevance to understanding and treating depression. Pharmacol Ther 132(3): 242-267.
14. Vollmayr B, Bachteler D, Vengeliene V, Gass P, Spanagel R, et al. (2004) Rats with congenital learned helplessness respond less to sucrose but show no deficits in activity or learning. Behav Brain Res 150(1-2): 217-221.
15. Dalla C, Edgecomb C, Whetstone WS, Shors TJ (2008) Females do not express learned helplessness like males do. Neuropsychopharmacology 33(7): 1559-1569.
16. Tarr AJ, Wohleb ES, Godbout JP, Mo X, Sheridan JF, et al. (2013) Prolonged restraint stress increases IL-6, reduces IL-10, and causes persistent depressive-like behavior that is reversed by recombinant IL-10. PLoS One 8(3): e58488.
17. Willner P (2005) Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. Neuropsychobiology 52(2): 90-110.
18. Mutlu O, Gumuslu E, Ulak G, Celikyurt IK, Kokturk S, et al. (2012) Effects of fluoxetine, tianeptine and olanzapine on unpredictable chronic mild stress-induced depression-like behavior in mice. Life Sci 91(25-26): 1252-1262.
19. Golden SA, Covington HE 3, Berton O, Russo SJ (2011) A standardized protocol for repeated social defeat stress in mice. Nat Protoc 6(8): 1183-1191.
20. Graeff FG, Guimarães FS (2012) Fundamentals of psychopharmacology. 2nd ed. São Paulo: Ateneu.
21. American Psychiatric Association. Diagnostic and statistical manual of mental disorders - DSM-IV-TR®. 4th ed. Arlington: American Psychiatric Publishing.
22. Kessler RC, Demler O, Frank RG, Olfson M, Pincus HA, et al. (2005) Prevalence and treatment of mental disorders, 1990 to 2003. N Engl J Med 352(24): 2515-2523.
23. File SE (1992) Behavioural detection of anxiolytic action. In: Elliott JM, Heal DJ, Marsden CA, editors. Experimental approaches to anxiety and depression. New York: Wiley: 25-44.
24. Pellow S, File SE (1986) Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. Pharmacol Biochem Behav 24(3): 525-529.
25. Shepherd JK, Grewal SS, Fletcher A, Bill DJ, Dourish CT (1994) Behavioural and pharmacological characterisation of the elevated "zero-maze" as an animal model of anxiety. Psychopharmacology (Berl) 116(1): 56-64.
26. Viana MB, Tomaz C, Graeff FG (1994) The elevated T-maze: a new animal model of anxiety and memory. Pharmacol Biochem Behav 49(3): 549-554.
27. Teixeira RC, Zangrossi H, Graeff FG (2000) Behavioral effects of acute and chronic imipramine in the elevated T-maze model of anxiety. Pharmacol Biochem Behav 65(4): 571-576.
28. Crawley J, Goodwin FK (1980) Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. Pharmacol Biochem Behav 13(2): 167-170.
29. File SE, Wardill AG (1975) Validity of head-dipping as a measure of exploration in a modified hole-board. Psychopharmacologia 44(1): 53-59.

30. File SE, Hyde JR (1978) Can social interaction be used to measure anxiety? Br J Pharmacol 62(1): 19-24.

31. Cryan JF, Sweeney FF (2011) The age of anxiety: role of animal models of anxiolytic action in drug discovery. Br J Pharmacol 164(4): 1129-1161.

32. Bodnoff SR, Suranyi-Cadotte B, Quirion R, Meaney MJ (1989) A comparison of the effects of diazepam versus several typical and atypical anti-depressant drugs in an animal model of anxiety. Psychopharmacology (Berl) 97(2): 277-279.

33. Francis DD, Meaney MJ (1999) Maternal care and the development of stress responses. Curr Opin Neurobiol 9(1): 128-134.

34. Kasuga S, Ushijima M, Morihara N, Itakura Y, Nakata Y (1999) [Effect of aged garlic extract (AGE) on hyperglycemia induced by immobilization stress in mice] Nihon Yakurigaku Zasshi 114(3): 191-197.

35. Padovan CM, Guimarães FS (2000) Restraint-induced hypoactivity in an elevated plus-maze. Braz J Med Biol Res 33(1): 79-83.

36. Weber EM, Dallaire JA, Gaskill BN, Pritchett-Corning KR, Garner JP (2017) Aggression in group-housed laboratory mice: Why can't we solve the problem? Lab Anim 46(4): 157-161.

37. Koolhaas JM, Coppens CM, de Boer SF, Buwalda B, Meerlo P (2013) The resident-intruder paradigm: A standardized test for aggression, violence and social stress. J Vis Exp (77): e4367.

38. Terranova ML, Laviola G, de Acetis L, Alleva E (1998) A description of the ontogeny of mouse agonistic behavior. J Comp Psychol 112(1): 3-12.

39. Lindzey G, Winston H, Manosevitz M (1961) Social dominance in inbred mouse strains. Nature 191: 474-476.

40. Kwiatkowski CC, Akaeze H, Ndlebe I, Goodwin N, Eagle AL, et al. (2021) Quantitative standardization of resident mouse behavior for studies of aggression and social defeat. Neuropsychopharmacology 46(9): 1584-1593.

41. Matzel LD, Kolata S, Light K, Sauce B (2017) The tendency for social submission predicts superior cognitive performance in previously isolated male mice. Behav Processes 134: 12-21.

42. Matsumoto K, Pinna G, Puia G, Guidotti A, Costa E (2005) Social isolation stress-induced aggression in mice: a model to study the pharmacology of neurosteroidogenesis. Stress 8(2): 85-93.

43. Hashikawa K, Hashikawa Y, Falkner A, Lin D (2016) The neural circuits of mating and fighting in male mice. Curr Opin Neurobiol 38: 27-37.

44. Mikami K, Watanabe N, Tochio T, Kimoto K, Akama F, et al. (2023) Impact of gut microbiota on host aggression: Potential applications for therapeutic interventions early in development. Microorganisms 12: 1008.

45. Mortimer N, Ganster T, O'Leary A, Popp S, Freudenberg F, et al. (2019) Dissociation of impulsivity and aggression in mice deficient for the ADHD risk gene Adgrl3: Evidence for dopamine transporter dysregulation. Neuropharmacology 156: 107557.

46. Jhun M, Panwar A, Cordner R, Irvin DK, Veiga L, et al. (2020) CD103 deficiency promotes autism (ASD) and attention-deficit hyperactivity disorder (ADHD) behavioral spectra and reduces age-related cognitive decline. Front Neurol 11: 557269.

47. Moy SS, Nadler JJ, Perez A, Barbaro RP, Johns JM, et al. (2004) Sociability and preference for social novelty in five inbred strains: An approach to assess autistic-like behavior in mice. Genes Brain Behav 3(5): 287-302.

48. McFarlane HG, Kusek GK, Yang M, Phoenix JL, Bolivar VJ, et al. (2008) Autism-like behavioral phenotypes in BTBR T+tf/J mice. Genes Brain Behav 7(2): 152-163.

49. Pearson BL, Pobbe RLH, Defensor EB, Oasay L, Bolivar VJ, et al. (2011) Motor and cognitive stereotypies in the BTBR T+tf/J mouse model of autism. Genes Brain Behav 10(2): 228-235.

50. Reynolds S, Urruela M, Devine DP (2013) Effects of environmental enrichment on repetitive behaviors in the BTBR T+tf/J mouse model of autism. Autism Res 6(5): 337-343.

51. Laucht M, Esser G, Baving L, Gerhold M, Hoesch I, et al. (2000) Behavioral sequelae of perinatal insults and early family adversity at 8 years of age. J Am Acad Child Adolesc Psychiatry 39(10): 1229-1237.

52. Mcintosh DE, Mulkins RS, Dean RS (1995) Utilization of maternal perinatal risk indicators in the differential diagnosis of ADHD and UADD children. Int J Neurosci 81(1-2): 35-46.

53. Holtzman DM, Morris JC, Goate AM (2011) Alzheimer's disease: the challenge of the second century. Sci Transl Med 3(77): 77sr1.

54. Jiang T, Yu JT, Tian Y, Tan L (2013) Epidemiology and etiology of Alzheimer's disease: from genetic to non-genetic factors. Curr Alzheimer Res 10(8): 852-867.

55. Langbaum JB, Fleisher AS, Chen K, Ayutyanont N, Lopera F, et al. (2013) Ushering in the study and treatment of preclinical Alzheimer disease. Nat Rev Neurol 9(7): 371-381.

56. Wang Q, Xu Z, Tang J, Sun J, Gao J, et al. (2013) Voluntary exercise counteracts Aβ25-35-induced memory impairment in mice. Behav Brain Res 256: 618-625.

57. Chen H, Yang Y, Yao HH, Tang XC, Ding JH, et al. (2006) Protective effects of iptakalim, a novel ATP-sensitive potassium channel opener, on global cerebral ischemia-evoked insult in gerbils. Acta Pharmacologica Sinica 27(6): 665-672.

58. Lo SC, Scearce-Levie K, Sheng M (2016) Characterization of social behaviors in caspase-3 deficient mice. Sci Rep 6: 18335.

59. Elbaz A, Carcaillon L, Kab S, Moisan F (2016) Epidemiology of Parkinson's disease. Rev Neurol 172(1): 14-26.

60. Obeso JA, Rodriguez-Oroz MC, Goetz CG, Marin C, Kordower JH, et al. (2010) Missing pieces in the Parkinson's disease puzzle. Nat Med 16(6): 653-661.

61. Jankovic J (2008) Parkinson's disease: clinical features and diagnosis. J Neurol Neurosurg Psychiatr 79(4): 368-376.

62. Schilder JC, Overmars SS, Marinus J, Van Hilten JJ, Koehler PJ (2017) The terminology of akinesia, bradykinesia and hypokinesia: Past, present and future. Parkinsonism Relat Disord 37: 27-35.

63. Perlmutter JS (2009) Assessment of Parkinson disease manifestations. Curr Protoc Neurosci Chapter 10:1.

64. Seibenhener ML, Wooten MC (2015) Use of the Open Field Maze to measure locomotor and anxiety-like behavior in mice. J Vis Exp (96): e52434.

65. Fleming SM, Zhu C, Fernagut PO, Mehta A, Dicarlo CD, et al. (2004) Behavioral and immunohistochemical effects of chronic intravenous and subcutaneous infusions of varying doses of rotenone. Exp Neurol 187(2): 418-429.

66. Matsuura K, Kabuto H, Makino H, Ogawa N (1997) Pole test is a useful method for evaluating the mouse movement disorder caused by striatal dopamine depletion. J Neurosci Methods 73(1): 45-48.

67. Dunham NW, Miya TS (1957) A note on a simple apparatus for detecting neurological deficit in rats and mice. J Am Pharm Assoc Am Pharm Assoc 46(3): 208-209.

68. Brooks SP, Dunnett SB (2009) Tests to assess motor phenotype in mice: a user's guide. Nat Rev Neurosci 10(7): 519-529.

69. Deacon RM (2013) Measuring motor coordination in mice. J Vis Exp (75): e2609.

70. Gonzalez-Latapi P, Bayram E, Litvan I, Marras C (2021) Cognitive impairment in Parkinson's disease: epidemiology, clinical profile, protective and risk factors. Behav Sci (Basel)11(5): 74.

71. Degirmenci Y, Angelopoulou E, Georgakopoulou VE, Bougea A (2023) Cognitive impairment in Parkinson's disease: an updated overview focusing on emerging pharmaceutical treatment approaches. Medicina 59(10): 1756.

72. Ballard CG, Aarsland D, Mckeith I, O'brien J, Gray A, et al. (2002) Fluctuations in attention: PD dementia vs DLB with parkinsonism. Neurology 59(11): 1714-1720.

73. Kehagia AA, Barker RA, Robbins TW (2010) Neuropsychological and clinical heterogeneity of cognitive impairment and dementia in patients with Parkinson's disease. Lancet Neurol 9(12): 1200-1213.

74. Levin BE (1990) Spatial cognition in Parkinson disease. Alzheimer Dis Assoc Disord 4(3): 161-170.

75. Matison R, Mayeux R, Rosen J, Fahn S (1982) "Tip-of-the-tongue" phenomenon in Parkinson disease. Neurology 32(5): 567-570.

76. Lee CS, Cenci MA, Schulzer M, Bjorklund A (2000) Embryonic ventral mesencephalic grafts improve levodopa-induced dyskinesia in a rat model of Parkinson's disease. Brain 123(Pt 7): 1365-1379.

77. Laatu S, Revonsuo A, Pihko L, Portin R, Rinne JO (2004) Visual object recognition deficits in early Parkinson's disease. Parkinsonism Relat Disord 10(4): 227-233.

78. Morris RG, Garrud P, Rawlins JN, O'keefe J (1982) Place navigation impaired in rats with hippocampal lesions. Nature 297(5868): 681-683.