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A short review on behavioural assessment methods in rodents

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Abstract:
The rodent behavioural examination techniques are used to assess various psychological, neurological models and neurotoxicity studies. Therefore, it is of interest to document the various behavioural assessment methods used in rodent model to study the motor, sensory, cognitive functions and emotional behaviour.

Key Words: Rodents, behavioural, cognitive, motor, social activities

Background:
Over the past decade, there has been a steady increase in the study of laboratory animal behavior. This field has placed a growing emphasis on various commonly used species, recognizing the significance of animals' motor, cognitive, and social activities**. [1]** Despite being kept in captivity for extended periods, laboratory animals retain their instinctual behaviors and characteristics that evolved in their wild ancestors. It is essential to test their natural active behaviors, such as exploration, inquisitiveness, and digging, aggression, rearing, and climbing, while considering well-defined circadian rhythms**. [2]** Behavior assessment is a scientific approach that relies on the ability of human observers to integrate observed details of behavior, posture, and context to summarize animals' behavioral patterns. [**3**] Observers use descriptors like "relaxed," "anxious/tense," "frustrated," or "content" to evaluate and assess animals' emotional states. The use of feasible indicators to evaluate animals' emotional states is strongly recommended in behavioral assessments. **[4]** Behaviors can be measured in terms of interval (also known as latency), frequency, and duration. Interval measures the time it takes for a specific behavior to occur. It can be measured in seconds, minutes, or hours. Frequency refers to the number of times a behavior occurs during an observation period and is usually measured per minute, per hour, or per day. Duration measures how long a behavior lasts and can be measured in seconds, minutes, or hours. **[5]** Rodent models are essential for studying brain disorders, including neurodevelopmental, neuropsychiatric, and neurodegenerative diseases. They help increase our understanding of underlying pathology and serve as preclinical models for testing potential treatments. **[6]** Behavioral outcomes are among the most significant measures in these studies. Unfortunately, reports from different laboratories often yield conflicting results, and findings from rodent models are not consistently replicated in human trials **[7].** There are several well-established tests available to assess various behavioural readouts. However, subtle aspects such as housing conditions, testing conditions, and the sex and strain of animals can influence the measurements. **[8]** Therefore, it is important to consider these factors during behavioral testing to ensure the reliability and reproducibility of results. Zhang *et al.* in 2017 used a chronic migraine rat model to evaluate depression and anxiety behavior using a panel of tests. **[9]** The group that received inflammatory soup infusion showed a decrease in sucrose preference, locomotor and rearing behaviors, inner zone distance percent, open-arm entry percent, and serotonin & dopamine levels in the prefrontal brain**. [10]** Weight, inner zone time percentage, or open-arm time percentage weren't different between the two groups. Therefore, it is of interest to document the various behavioural assessment methods used in rodent model to study the motor, sensory, cognitive functions and emotional behaviour. cognitive and behavioral in rodent models of brain aging, dementia and various behavioral paradigms, such as the Y maze, Morris water maze, Barnes maze, and more, were discussed. These paradigms are used to test spatial memory, recognition memory, semantic memory, spatial memory, and emotional memory **[11].**

Tests to assess the motor function and coordination:
Table 1 presents various behavioral tests for evaluating motor function and coordination. Tests include the staircase, beam walking, rotarod, open field, cylinder, foot-fault, pole, and elevated body swing tests for muscle strength. Evaluation of fine motor coordination and balance involved tests like staircase, rotarod, cylinder, grid walk, and beam walking. Tests such as forelimb placing identified forelimb function and deficits, while the wire hanging test evaluated locomotor abnormalities and behavioral deficits **[12].** The climbing test assessed motor impairments, and the grid stepping evaluated sensorimotor deficits. Grip strength was measured to assess skeletal muscle function, and skilled limb use evaluated voluntary motor control. Reaching tests and the acoustic startle response assessed motor and cognitive performance. Operant tasks were used for cognitive performance evaluation **[13].** Tests like the rotarod, triple horizontal bar, static rods, and parallel bar evaluated muscle coordination and strength **[14].** Foot fault assessed motor functions, and the pole test evaluated movement disorders **[9].** Neuromuscular weakness was assessed through grip strength, swimming tests measured endurance, and gait analysis identified ataxic and paretic gait. Various methods were applied for assessing motor coordination, balance, and rigidity **[15].**

Table 1: List of various behavioural tests to assess the motor function and coordination

Name of the Behavioral Test	To Identify
Beam Walking	Motor Coordination

Rota rod	Coordination of Muscle And Balance
Open Field	Locomotion
Elevated Body Swing	Strength of the Muscle
Cylinder Test	Motor Coordination
Flexion of Forelimb	Forelimb Function
Placing of Forelimb	Forelimb Function and Slippage
Grid Walking	Motor Coordination and Placing Deficits During Locomotion
Ledged Tapered Beam	Hind Limb Functioning
Reaching Chamber/Pellet Retrieval	Skilled Forepaw Use and Motor Functioning
Staircase	Forelimb Extension, Grasping Skills, Side Bias and Independent Use of Forelimbs
Pasta	Manual Dexterity and Fine Motor Skills
Ladder Rung Walking	Fore- and Hind Limb Stepping, Placing And Coordination
Wire Hanging	Abnormalities in Locomotion and behavioral Deficits in Models
Horizontal Ladder	Walking Ability
Swimming	Coordinated Limb Use During Voluntary Locomotion
Climbing	Monitor Motor Impairments
Grid Stepping	Assess of Sensoromotor Deficit
Skilled Limb Use	Analyze Finer Aspects of Voluntary Motor Control
Descriptive Paw Use	Characterization of Fine Motor Control
Reaching Tests	Skilled Forepaw Use and Motor Functioning
Acoustic Startle Response	Muscular Activity Produced in Response to a Sudden Loud Sound
Operant Tasks	Motor and Cognitive Performance in Rats and Mice
Sequence Learning	Motor Habit Learning Task
Triple Horizontal Bar	Measure Motor Coordination and Strength
Static Rods	Measure Motor Coordination
Parallel Bars	Measuring Motor Capabilities
Foot-Fault Test	Assess Motor Functions
Pole Test	Assess Movement Disorders in Mice
Grip Strength	Neuromuscular Weakness
Gait Analysis	Ataxic and Paretic Gait
Treadmill, Coordinated Movement Test	In coordination
Bar Test	Catalepsy Bar Test to Measure Rigidity

Table 2 List of specific methods in testing sensory function of rodents.

Name of the Behavioural Test	To Identify
Limb Placement Test	Sensorial Limb Placement
Corner	Sensorimotor and Postural Asymmetries - Whiskers Sensitivity
Adhesive Removal Test	Tactile Responses and Asymmetries
Whisker Nuisance Task	Sensorimotor Integration
Gap Cross Test	Evaluation of Somatosensory Behaviour
Angle Entrance Task	Evaluation of Somatosensory Behaviour
Whisker Guided Exploration Task	Somatosensory Behavior
The Von Frey Hair Test	Evaluate Sensory Function
Sticky Dot Test	Assess Sensorimotor Deficits
Chemical Stimuli:	
The Formalin Pain Test	Biphasic Pain Response
Mechanical Stimuli:	
Manual or Electronic Von Frey Test	Stimulus Intensity that Evokes A Withdrawal Reflex
The Randall Selitto Test	Measurement of Pain Response
Heat Stimuli:	
Tail Flick Test	Test for Pain Response
Hot Plate Test	Test for Pain Response
Hargreaves Test	Assess Thermal Pain Sensation
Thermal Probe	Test to Quantify Heat Thresholds
Cold Stimuli:	
Cold Plate Test	Understanding Cold Allodynia and Hyperalgesia
Acetone Evaporation	Test to Cold Allodynia
Cold Plantar Assay	Assessment of Cold Sensitivity
Tests For Escape Behavior:	
Paw Withdrawal Test	Pain Leading to Escape Reaction
Acoustic Startle	Reflex Response to Sudden Loud Sound
Sensorimotor Gating	Pre-pulse Inhibition- Weak Stimulus Suppress the Startle Response

Table 3: List of various methods in testing cognitive (learning and memory) functions of rodents

Name of the Behavioural Test	To Identify
Morris Water Maze Test	Spatial Learning and Memory
Radial Arm Maze Test	
Modified Elevated Plus-Maze Test	Spatial Learning
Three-Panel Runway Maze	Working Memory
Y-Maze Test	Active Working Short-Term Memory
Barnes Maze	Working and Spatial Memory Acquisition

T-Maze Test	Acquistion, Short-Term Memory, Learning
8-Arm Radial Maze Test	Short-Term and Spatial Memory Testing
Y Maze Forced Alternation Task	Exploratory Behaviour and Working Memory
Radial Arm Water MazeTest	Spatial and Working Reference Memory
Y-Maze Spontaneous Alternation Task	Spatial Working Memory, Learning to Explore New Environment
Star Maze	Spatial Learning
Cincinnati Water Maze	Measuring Escape Latency
Multiple T-Maze	Memory and Spatial Learning
Complex Alley Maze	Exploratory, Learning Behaviour
Cheeseboard Maze	Assess Spatial Learning and Memory
Hole Board Discrimination Test	Spatial Working and Reference Memory Performance
Operant Chamber	Learned Performance Short-Term Memory
Passive Avoidance Test	Avoidance Learning
Novel Object Recognition Tests	Associative Memory, Recognition Memory, Declarative Memory, Working Memory
Swimming Pool Spatial Tasks	Spatial Behavior
Fear Conditioning	Associative Memory
Hippocampal Working Memory Test	Spontaneous Alternation in Y-Maze
Olfactory Learning and Memory Test	Conditioned Preference for Odor

Table 4 List of various methods in assessing emotional state of rodents.

Tests to Evaluate Emotion, Anxiety and Depression like Behavior:	
Open Field	Anxiety and Exploratory Activity
Novelty Suppressed Feeding	Measurement of Depression like Behaviours
Elevated Plus Maze	Measurement of Depression like Behaviours
Light/Dark Box	Measurement of Anxiety Related Behavior
Stress Induced Hyperthermia	Screening for Anxiolytic Potential in Test Compound
Elevated Zero Maze	Measurement of Anxiety Related Behavior
Marble Burying Test	Measurement of Anxiety Related Behavior
Hole-Board Test	Measurement of Anxiety, Stress And Emotionality
Sucrose Preference Test	Indicator of Anhedonia
Tail Suspension Test	Measurement of Stress
Forced Swim Test	Models of Depressive like Behavior
Coated Test	Decreased Self-Care - Depression-like Behavior
Splash Test	Reduced Grooming Behavior - Depression
Nesting Test	Assessing Behavior Associated with Depression

Tests to assess sensory function:

Table 2 outlines methods for testing sensory functions in rodents, including sensory assessment and sensorimotor behavior. Tests for sensory neglect and sensorial limb placement comprised the adhesive removal and limb placement tests. The corner test evaluated sensorimotor and postural asymmetries, while adhesive removal identified tactile responses and asymmetries **[9,16].** Mechanical allodynia measured pain response, and thermal hyperalgesia assessed heat thresholds. Cold hyperalgesia tests were conducted for cold allodynia and hyperalgesia **[12].** Von Frey hair and sticky dot tests were employed for sensory and sensorimotor function evaluation **[7].** Chemical stimuli were evaluated using the formalin pain test, hot and cold temperature tests and mechanical stimuli were measured through the Von Frey and Randall Selitto tests. **[17]** Heat stimuli were assessed with tail flick and hot plate tests, and cold stimuli with cold plate and acetone evaporation tests. Paw withdrawal, acoustic startle, and sensorimotor gating tests assessed escape behavior **[14].**

Test to assess cognitive (learning and memory) functions:

Table 3 lists methods for testing cognitive (learning and memory) functions of rodents. Tests included the Morris water maze, radial arm maze, and modified elevated plus-maze for spatial learning and memory. Passive avoidance and the three-panel runway maze assessed avoidance learning and working memory **[18].** The Y-maze test evaluated active working short-term memory, while the object recognition test assessed various memory types **[16].** Barnes maze, eight-arm radial maze, and swimming pool spatial tasks were used for memory acquisition assessment **[13].** T-maze and operant chamber tests assessed short-term memory and learned performance **[14].** Radial arm water maze and Y-maze spontaneous alternation task evaluated spatial working memory **[11].** Novel object recognition and fear conditioning assessed associative and working memory **[19].** Olfactory memory and learning were measured through odor preference conditioning **[14].**

Test to assess emotional state:

Table 4 provides methods for assessing the emotional state of rodents, including anxiety and depression symptoms. Tests for anxiety-like behavior encompassed open field, novelty suppressed feeding, and raised plus maze tests. The light/dark box test evaluated anxiety-related behavior **[20].** Elevated zero maze and marble burying tests were employed for anxiety-related behaviors, and the hole-board test measured anxiety, stress and emotion. Tests to assess emotion and depression included the forced swim test, sucrose preference, and tail suspension test **[7].** Coated and splash tests evaluated depression-like behavior. Upraised plus maze and forced swim tests assessed depression **[21].** Tests such as the star maze and hole-board maze were used to measure emotional state **[15].** Novelty-suppressed feeding and forced swim tests were employed for depression assessment **[7].**

Conclusion:

Behavioural assessment is a critical parameter in neurotoxicity studies, drug development, and neurological and psychological model screening. A wide range of tests are available for assessing motor, sensory, cognitive, and emotional states in rodents. The choice of tests should align with the study's nature and be carried out with adherence to ethical animal handling practices. To minimize bias during analysis, video recordings of behavioral sessions are recommended.

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