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RESEARCH ARTICLE

Behavioral Paradigms in Rodent Models of Amyotrophic Lateral Sclerosis

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) remains an untreatable neurodegenerative disease without a cure or effective treatment, mainly due to elusive underlying mechanisms. ALS is primarily characterized by motor neuron dysfunction in the brain and spinal cord. However, it also exhibits non-motor symptoms such as executive, behavioral, and language dysfunction, making it challenging to establish informative disease models for relevant preclinic and clinical research. The discovery of ALS-causing genes has paved the way for the development of various animal models. Among these models, rodents have emerged as particularly valuable, demonstrating unique ALS-related behavioral defects in multiple behavioral tests. These models enable further understanding of disease mechanisms and provide sensitive and precise functional assessments for drug development. Given the intricate nature of ALS pathology, it is crucial and challenging to select appropriate behavioral tests as functional exploratory readouts, mainly due to the diverse array of ALS rodent models exhibiting distinct behavioral paradigms. Therefore, this report endeavors to present an overview of various behavioral assessments, encompassing motion ability tests, cognitive evaluations, sensory analyses, and other paradigms described in rodent models of ALS. Our goal is to summarize and compare the behavioral alterations observed in diverse rodent models of ALS with distinct gene mutations, thus providing comprehensive references and guidance for advancing pathogenic and therapeutic research in ALS.

Keywords: Behavioral paradigms, Amyotrophic lateral sclerosis, SOD1, Motor defect

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease that impacts both upper and lower motor neurons within the central nervous system (CNS). Patients who have ALS also experience progressive muscle atrophy, ultimately resulting in paralysis and death within 3 to 5 years after disease onset. Unfortunately, there remains a significant lack of effective therapeutic approaches capable of halting, delaying, or reversing ALS in humans.

The identification of ALS-causing genes, such as *C9ORF72* (chromosome 9 open reading frame 72) and *SOD1* (superoxide dismutase 1), has led to the development of various animal models, including primates, rodents, zebrafish, and flies. These models have been established to mimic specific cellular and behavioral defects observed in ALS patients, facilitating in-depth investigations into disease mechanisms. Rodents have proven to be especially valuable among these models, as they manifest behavioral deficits unique to ALS through various behavioral tests.

However, the genetic landscape of ALS has expanded significantly in the past two decades with the identification of several more genes responsible for the disease. This augmented genetic diversity has introduced a layer of intricacy into behavioral phenotyping. The models employed often manifest a spectrum of abnormalities, most influenced by factors such as the specific gene, mutation, or promoter utilized for genetic manipulation. [1] Consequently, determining appropriate tests to assess disease progression and associated behavioral changes has become a formidable challenge.

To uncover the intricate mechanism underlying ALS, precise functional assessments and sensitive behavioral tests in animal models are imperative. This review provides a chronological overview of the diverse behavioral tests and methodologies employed in rodent models. We aim to elucidate the behavioral alterations that occur in rodent models with distinct gene mutations, thereby contributing to our understanding of ALS pathogenesis.

2. Motion Ability

As a progressive neurodegenerative disorder, ALS primarily affects motor neurons in the brain and spinal cord. This leads to the gradual weakening of voluntary muscles, affecting essential activities such as walking, grasping objects, and speaking. Consequently, numerous studies are dedicated to exploring the motion abilities of ALS patients, with a focus on identifying potential therapeutic interventions. Motor behavioral performance serves as a primary outcome measure in these investigations. Therefore, our initial objective is to provide a comprehensive summary of relevant behavioral metrics employed in rodent models of ALS (Table 1).

Table 1. Behavioral paradigms in ALS rodent models

References	Rodent Model	Behavioral analysis
SOD1		
[2]	hSOD1(G93A) Transgenic mouse	OFT, Survival test
[3]	hSOD1(G93A) Transgenic mouse	OFT
[4]	hSOD1(G93A) Transgenic mouse	OFT, Grip strength assessment of hindlimbs, Rota-rod test, Survival test
[5]	hSOD1(G93A) Transgenic mouse	OFT, Barnes maze test
[6]	hSOD1(G93A) Transgenic mouse	OFT, Survival test
[7]	hSOD1(G93A) Transgenic mouse	Gait analysis
[8]	hSOD1(G93A) Transgenic mouse	Gait analysis
[9]	hSOD1(G93A) Transgenic mouse	Gait analysis
[10]	hSOD1(G93A) Transgenic mouse	Gait analysis
[11]	hSOD1(G93A) Transgenic mouse	Gait analysis, Rota-rod test
[12]	hSOD1(G93A) Transgenic mouse	Gait analysis
[13]	hSOD1(G93A) Transgenic mouse	OFT, Grip strength assessment of hindlimbs
[14]	hSOD1(G93A) Transgenic rat	Grip strength assessment of hindlimbs, Survival test
[15]	hSOD1(G93A) Transgenic mouse	Grip strength assessment of hindlimbs
[16]	hSOD1(G93A) Transgenic mouse	Grip strength assessment of hindlimbs
[17]	hSOD1(G93A) Transgenic mouse	Grip strength assessment of hindlimbs
[18]	hSOD1(G93A) Transgenic mouse	Grip strength assessment of hindlimbs, Survival test, Rota-rod test

References	Rodent Model	Behavioral analysis
[19]	hSOD1(G93A) Transgenic mouse	Grip strength assessment of hindlimbs, Survival test
[20]	hSOD1(G93A) Transgenic mouse	Grip strength assessment of hindlimbs, Survival test, Rota-rod test
[21]	hSOD1(G93A) Transgenic mouse	Grip strength assessment of hindlimbs
[22]	hSOD1(G93A) Transgenic mouse	Rota-rod test, Survival test, Gait analysis
[23]	hSOD1(G93A) Transgenic mouse	Rota-rod test, Survival test
[24]	hSOD1(G93A) Transgenic mouse	Rota-rod test, Grip strength assessment of hindlimbs
[25]	hSOD1(G93A) Transgenic mouse	Rota-rod test, Grip strength assessment of hindlimbs, Gait analysis
[26]	hSOD1(G93A) Transgenic mouse	Rota-rod test
[27]	hSOD1(G93A) Transgenic mouse	Survival test, Neurological scoring system
[28]	hSOD1(G93A) Transgenic mouse	Grip strength assessment of hindlimbs, Survival test, Neurological scoring system
C9ORF72		
[29]	C9orf72$(G4C2)_{66}$ mouse	OFT
[30]	C9orf72$(G4C2)_{500}$ mouse	Morris water maze
TDP43		
[31]	TDP-43^{Q331K} knock-in mouse	Gait analysis
[32]	hTDP-43 Transgenic mouse	Gait analysis
[33]	hTDP-43 Transgenic mouse	Novel object recognition test, Social interaction test
FUS		
[34]	FUS Transgenic mouse	Gait analysis, Survival test
VPS54		
[35]	Wobbler mouse	Rota-rod test

2.1 OPEN FIELD TEST

The open field test (OFT), originally devised in 1934, remains a prevalent method for assessing changes in motor function in rodents. This test is characterized by its simplicity and efficiency, offering a rapid means of gathering a wide array of behavioral data, encompassing general ambulatory performance and insights into emotional states. [36] Numerous variables pertaining to various motor activities can be assessed and quantified within the OFT, including metrics such as total walking distance, zone entries, and the duration spent within predefined zones in the OFT maze. [37]

An intronic G4C2 hexanucleotide repeat expansion (HRE) within the *C9ORF72* gene is the predominant genetic cause of ALS. Emerging evidence suggests that the toxicity stemming from HREs plays a pivotal role in the development of ALS. A prior investigation uncovered that introducing G4C2 repeat expansions via an adeno-associated viral vector in a mouse model led to specific behavioral alterations within the open field test. Remarkably, mice with an increased number of G4C2 repeats displayed a reduced tendency inclination to explore the central area of the open field. On the other hand, these mice showed increased levels of hyperactivity in the open field, covering greater distances at an accelerated pace. [29] This heightened activity level bears relevance to the behavioral disinhibition frequently observed in neurodegenerative diseases. [38]

Transgenic mice expressing a G93A mutant form of human SOD1 (hSOD1(G93A)) have been a commonly used model for evaluating future therapeutic approaches in ALS research since 1994. [2] Notably, the early motor symptoms exhibited by SOD1 mutant rodents can be detected through the OFT, underscoring the feasibility and promise of using the OFT to identify subtle behavioral changes in ALS models. A distinctive motor pattern distinguishing SOD1 mutants from their wild-type counterparts emerged as early as 2 months prior to disease onset under some strain backgrounds. This pattern involved pronounced braking when moving near the arena wall, followed by a quick change in direction away from it. Two independent studies showed that SOD1 mutants exhibited this pattern significantly less frequently than wild-type controls. [3] For example, hSOD1(G93A) transgenic mice demonstrated significant deficits across various open-field measures compared to control mice, starting from 45 days of age. These measures included average locomotion velocity, ambulatory distance, rearing episodes, and resting time. [4] Furthermore, considering that the onset of tremors in one or both hindlimbs constitutes the first clinical symptoms of motor neuron disease (MND), [39] the motor function data revealed that motor performance deficits manifested several weeks prior to the onset of clinical symptoms in pre-symptomatic hSOD1(G93A) transgenic mice. In

addition, mice carrying mutant SOD1 did display increased anxiety-like behaviors and signs of memory dysfunction.[5] Utilizing the OFT, researchers successfully demonstrated the effectiveness of xenotransplantation of human umbilical cord blood mononuclear cells genetically modified with adenoviral vectors encoding *VEGF* (vascular endothelial growth factor) gene in transgenic hSOD1(G93A) mice. This finding holds promise as a potential therapeutic strategy for ALS.[6]

The mislocalization and aggregation of TDP-43 (TAR DNA-binding protein 43) is believed to be another contributing factor to the development of ALS. Researchers have devised a novel transgenic mouse model featuring human wild-type TDP-43 protein (hTDP-43) overexpression, specifically in forebrain neurons.[33,40,41] Initially, Alfieri and colleagues[41] observed the distance traveled and relative center distance in young transgenic hTDP-43 mice during a behavioral task closely resembled those of the control group. This similarity indicated the absence of motor or exploratory abnormalities in the early stages of the ALS model. However, their research uncovered a time-dependent decline in motor behavior, with long-term hTDP-43 expression ultimately resulting in negative responses in the OFT.

Due to its consistency and non-invasive advantages, the OFT is frequently employed to explore ALS pathological mechanisms and further provide potential treatment targets. Cyanobacterial β-N-methylamino-L-alanine (BMAA) has been under scrutiny as a potential contributing factor in the development of ALS.[42] However, previous research presented conflicting findings regarding the OFT results of BMAA exposure in animal models. One study administered BMAA (500 mg/kg) to rats on postnatal day (PND) 2 and PND 5 and reported no significant short- or long-term behavioral effects.[43] However, another research group demonstrated that acute alterations, including impaired motor capacity and hyperactivity, were observed when BMAA was administered to neonatal rats during the peak of the brain growth spurt (PNDs 9–10).[44] This divergence in findings suggests that the timing of BMAA exposure during critical developmental periods may be a crucial determinant of its behavioral impact. The brain growth spurt phase is susceptible to xenobiotics, and disturbances during this phase can lead to lasting changes in adulthood.[45] Recent research further substantiated the potential neurotoxicity of BMAA. Locomotor activity, as measured by the number of lines crossed and the time spent stationary in OFT, was significantly reduced following BMAA exposure on PND 3, 4, and 5, with a slightly more pronounced effect observed in female rats than in males.[46] These behavioral similarities between BMAA exposure and early ALS symptoms suggest the potential of BMAA as a neurotoxin contributing to the development of ALS in humans.

Additionally, enhanced Rho/Rho-kinase (ROCK) signaling has been implicated in various disorders within the central nervous system, including epilepsy, anxiety-related behaviors, antinociception, and ALS. However, a study involving repeated intracranial microinjections of ROCK inhibitor Y-27632 did not impact spontaneous locomotor activity in the OFT. Instead, it enhanced limb-placing accuracy in the ladder rung walking test.[47] This suggests that the therapeutic effects of targeted pathway inhibitors may be confined to the injection site and may not exert widespread impact due to factors such as microenvironment dysfunction and the low blood-brain barrier (BBB) permeability. These factors should be considered when choosing specific behavioral tests to evaluate potential ALS treatments.

2.2 GAIT ANALYSIS

In human ALS patients, alterations in gait rhythm have been observed, characterized by increased stride-to-stride fluctuations in magnitude and decreased dynamic stability.[48-52] Gait analysis has become invaluable for recapitulating and evaluating behavioral phenotypes in animals that are observed in human patients.[53] Notably, a previous study revealed that the expression of mutant hTDP-43 throughout the CNS in mice led to progressive gait disturbances.[54] Another study employed a treadmill gait analysis system and found that transgenic hSOD1(G93A) mice, compared to their wild-type counterparts, exhibited significantly longer stride and stance times during walking.[7] Consistent with a prior study,[55] stance time emerged as a sensitive indicator of gait differences between transgenic hSOD1(G93A) mice and wild-type mice, with group differences becoming more pronounced at higher treadmill speeds.

Given the significance of gait analysis in evaluating functional performance and its adaptability to various recording environments, researchers have explored integrating gait analysis with different monitoring techniques. For example, Hadzipasic et al[8] conducted single-unit extracellular recordings within spinal cord motor neurons for hindlimb muscles. They simultaneously recorded electromyograms (EMGs) of the corresponding muscles in awake symptomatic-stage transgenic hSOD1(G93A) mice as they performed self-initiated walking on a wheel at PND 135.[8] Their findings indicated a reduction in instantaneous firing

frequency, primarily driven by the loss of the fastest-firing motor neurons. [56] These observations correlated with step-to-step variability in EMG signals and flexor-extensor coactivation. Further kinematic analysis of gait suggested that motor disability may result from abnormal motor firing patterns accompanied by alterations in EMG patterns.

Indeed, the feasibility and reliability of computerized gait analysis, especially on a motorized treadmill, for measuring motor deficits in the mouse model of ALS have been debated. One study reported that treadmill gait analysis failed to consistently distinguish hSOD1(G93A) transgenic mice from the controls between 6 to 12 weeks of age and did not detect early motor deficits in these mice. [9] However, other researchers have argued that gait alterations in ALS models can be effectively characterized using treadmill gait analysis[10,11] or an automated gait analysis system (CatWalk). [12] Recent research has also shed light on sex-related differences in ALS mouse models. One study found that female ALS mice exhibited less severe motor dysfunction compared to males when using the CatWalk system. At the same time, significant gait deficits were still observed in aged ALS females. [31] Interestingly, the study also indicated that functional motor units remained relatively well preserved in these female ALS mice, as evidenced by compound muscle action potentials (CMAP) measurements in the hindlimbs. These sex-related differences, while complex and influenced by various confounding factors, such as genetic backgrounds and transgene expression levels, [57,58] may offer some insights into why ALS is more commonly observed in men than in women. Understanding the nuances of motor deficits in ALS, including potential sex-based differences, is crucial for advancing our comprehension of the disease and developing more effective therapeutic strategies.

Additionally, motor deficits have been successfully identified through gait analysis in other ALS mouse models, demonstrating the versatility of this approach in studying the disease. For example, gait analysis has been applied to transgenic mouse models induced by the expression of pathogenic truncated forms of the human *FUS* (fused in sarcoma) gene[34] or by transgenic mice expressing full-length hTDP-43 under the control of the mouse prion promoter. [32] These findings further highlight the utility of gait analysis as a valuable tool for characterizing motor defects in various ALS models, contributing to our understanding of the disease and potential therapeutic interventions.

2.3 GRIP STRENGTH ASSESSMENT OF HINDLIMBS

In ALS, the progression of the disease primarily results in widespread muscle weakness and atrophy, which correlates with a decline in hindlimb muscle strength. Therefore, muscle strength is a critical outcome measure used in clinical trials to diagnose and assess ALS progression. [59-61] In rodent models, assessing grip strength provides a straightforward means of determining changes in muscle strength as ALS progresses. This assessment is often called the paw grip endurance (PaGE) test. In short, the animal is placed on top of a wire cage lid, which is lightly shaken three times to induce the mouse to grip the wires. Subsequently, the lid is inverted and held approximately 20 cm above a cage containing fresh bedding. The time it takes for the animal to fall off the wire lid is recorded as an indicator of grip strength. Additionally, the duration during which animals can sustain their weight while holding onto a suspended metal rail midair is recorded as another measure of hindlimb grip strength.

By detecting statistically significant grip strength differences between ALS and wild-type animals, researchers have demonstrated that hindlimb grip strength assessment in hSOD1(G93A) mice is a highly sensitive and repeatable motor behavioral test for detecting early onset ALS. [13] This test can effectively be applied even when working with small cohorts of animals compared to OFT and gait analysis. Interestingly, males and females exhibit distinct patterns of grip strength loss, with females displaying a similar onset of grip strength loss in both hind- and forelimbs. In contrast, males experience a significantly earlier decrease in hindlimb strength. [15,62] It is important to note that the choice of assessment method can substantially impact the results. Some researchers use methods such as the PaGE or hanging wire test to detect behavioral changes in transgenic hSOD1(G93A) mouse models. [16,63] However, these methods may be less effective in detecting early ALS onset than other techniques. For instance, hTDP-43 transgenic mice showed no change in latency to fall in a hang wire test, indicating intact grip strength. [33] However, with advances in technology, the use of digital gauges for quantitative collection and processing of grip strength data has enhanced the reliability of this phenotype as an early indicator of ALS progression.

Grip strength data also helps establish a correlation between phenotypical behavioral changes and alterations that occur at the molecular level. For instance, one study using hanging wire test, revealed that ALS significantly diminishes muscle strength and elevates oxidative stress markers in skeletal muscle, which were improved through swim

training. [17] Using hSOD1(G93A) transgenic mice, several studies have established connections between motor performance in grip strength tests and specific signaling pathways in motor neurons, such as EphA4[18] and PAGE. [19] These studies provide valuable evidence supporting therapeutic interventions targeting these pathways to slow disease progression. Grip strength assessments also serve to evaluate the therapeutic efficacy of candidate drugs for ALS. For example, daily treatment with protocatechuic acid (PCA) administered via oral gavage, beginning at the onset of the disease, significantly extended survival and preserved skeletal muscle strength, as evidenced by grip strength tests. [20] In a recent study, researchers observed that neurturin had dose-dependent neuroprotective effects on cervical motor neurons and neuromuscular junctions. These effects led to a deceleration in the decline of forelimb grip strength in ALS mice. [21]

However, it is important to note that not all studies find grip strength to be the most sensitive indicator of disease severity or drug effectiveness in treating ALS. One study reported that, compared to grip strength, parameters derived from the OFT, such as average locomotion velocity, ambulatory distance, rearing episodes, and resting behavior, were more sensitive to the impacts caused by overexpression of mutant SOD1G93A. [63] This suggests the importance of exercising caution before exclusively relying on grip strength as an indicator of ALS progression and therapeutic outcomes and that a combination of complementary behavioral tests may provide a more comprehensive evaluation.

2.4 ROTA-ROD TEST

The rota-rod test is a widely utilized assessment designed to evaluate motor coordination, balance, and motor learning in rodents. The animal is placed on a rotating rod, commonly known as the "Rota-rod" apparatus, which gradually increases in speed. The rod itself can take the form of either a cylindrical or flat surface, and its surface texture can be adjusted to influence the level of difficulty in the task. The latency to fall, which is the time it takes for the animal to fall off the rotating rod, for each trial will be recorded as the read-out.

The behavior exhibited by rodents on the rota-rod can be influenced by both upper motoneurons and cerebellar neurons in the brain and lower motoneurons in the spinal cord. This comprehensive evaluation assesses various aspects of motor function, including motor balance, motor learning, neuromuscular coordination, and muscle strength and stamina. [4]

The rota-rod test is categorized into two modes: constant speed and accelerating speed. However, a lack of standardization has led to variations in the specific parameters used across different laboratories. For instance, in one study, a constant speed of 10 rotations per minute (rpm) was maintained for 180 seconds. [22] In contrast, another study employed a constant speed of 15 rpm for 600 seconds. [23] Furthermore, numerous laboratories prefer an accelerating speed methodology in their rota-rod tests. For instance, in the work conducted by Yoo et al, [24] the rota-rod speed systematically escalated from 4 rpm to 40 rpm at 1 rpm increments, with each increment taking approximately 3 seconds. In another study, [25] they chose a different approach, maintaining a constant speed of 11 rpm for 180 seconds. On the other hand, Maria et al[35] adopted an accelerating rod featuring a speed range spanning from 1 to 40 rpm and a ramp-up period lasting 180 seconds. In contrast, Shulamit et al[26] employed a markedly different paradigm, using an accelerating rod with rotation speeds that gradually increased from 2.5 to 25 rpm over a 5-minute time frame. Subsequently, subjects were exposed to the maximal rotation speed of 25 rpm for an additional 3 minutes. Various laboratories utilize different settings, likely in order to more effectively observe functional defects in ALS models across diverse genetic or strain backgrounds. However, this poses challenges when attempting to consolidate mechanisms explored in various labs, underscoring the need for standardized protocols to comprehensively investigate this disease.

Despite these variances, researchers can effectively distinguish motor defects induced by ALS. As indicated by the symptom onset date, the responses to this test are significantly influenced by the choice of mouse strain for ALS studies rather than the specific tests employed. For instance, in the case of hSOD1(G93A) transgenic mice under a C57BL/6J background, motor defects typically manifest at around 130 days of age. [24,25] Conversely, motor defects become evident in hSOD1(G93A) transgenic mice under a B6SJL mixed background at approximately 90 days of age. [23]

It is essential to acclimate the mice to the rota-rod before conducting the actual tests, which helps ensure more consistent and meaningful results. However, this training process can be time-consuming, posing a challenge for studies focusing on early behavioral changes as the primary read-out. Additionally, the rotarod test may not possess the sensitivity required to detect subtle changes in motor function. Mice can quickly reach a peak performance level (referred to as a 'ceiling effect')

or be too impaired to execute the task (referred to as a 'floor effect'). These limitations can hinder the ability of rota-rod test to detect subtle variations in motor function that may be ameliorated by potential therapeutic interventions. These limitations significantly restrict the application of the rotarod assay in certain ALS studies.

3. Cognition and Sentiment

Although ALS is predominantly associated with motor dysfunction, it is essential to note that cortical damage also occurs. In this context, Frontotemporal Dementia (FTD) is recognized for its progressive degeneration of neurons in the brain. ALS and FTD share a common feature: the abnormal accumulation of a protein known as TDP-43 within affected nerve cells. As a result, they are classified under the umbrella of 'TDP-43 proteinopathies.' However, there are many cases in which these conditions intersect, giving rise to a condition known as ALS-FTD. In ALS-FTD, individuals may exhibit a combination of the motor symptoms typical of ALS and the behavioral changes distinctive to FTD. Notably, mutations in the *TARDBP* (TAR DNA binding protein) gene or *C9ORF72* gene are a shared genetic factor in both ALS and FTD. Therefore, to gain a comprehensive understanding of cognitive and emotional impairment in ALS, it is essential to review mouse behavior in FTD studies. ALS predominantly affects motor neurons, leading to muscle weakness, atrophy, and eventual paralysis. In contrast, FTD primarily impacts cognition, memory, social behavior, personality, and language.

3.1 NOVEL OBJECT RECOGNITION TEST

The novel object recognition test is a valuable method for assessing recognition memory in mice. The procedure involves multiple stages: the pre-training phase, the subsequent training phase, and the test phase. During the pre-training phase, animals are introduced into an empty arena for several sessions. In the subsequent training phase, mice are exposed to two identical objects positioned at opposite ends of the arena for 10 minutes. In the test phase, the mice are given the opportunity to explore one copy of the previously presented object (familiar) and a new object (novel) for 5 minutes. Researchers then record the time spent exploring each of these objects. [41] In a specific study, it was observed that wild-type mice exhibited a clear preference for the novel object. In contrast, this preference was absent in hTDP-43 transgenic mice, suggesting potential impairment in the functions of the perirhinal and prefrontal cortices. [33]

3.2 MORRIS WATER MAZE

Like hTDP-43 transgenic mice, mice overexpressing the *C9ORF72* mutation exhibit cognitive deficits that can be assessed using the Morris water maze. The Morris water maze consists of a large circular pool or tank filled with opaque water. Inside this pool, a concealed escape platform is positioned just beneath the water's surface in one of the quadrants. The animal's ability to locate this hidden platform during both the training and testing phases offers valuable insights into their spatial learning and memory abilities. In ALS mice, notable memory impairment was observed at 4.5 months of age despite the absence of apparent motor deficits. [30] This impairment was evidenced by an increased amount of time spent trying to escape from the water maze, reflecting the challenges they faced in memory-related tasks.

3.3 SOCIAL INTERACTION TEST

Social disinterest is a recurring characteristic observed in patients with FTD, [64] and this behavior can also be replicated in mouse models. To assess the social aspect of behavioral deficits in ALS rodent models, researchers employ a three-chamber social interaction test. This test measures the active interaction time of a test mouse with a novel probe mouse placed in a cylinder within one of the chambers, known as the "social side," or with an empty cylinder in the opposite chamber, designated as the "nonsocial side." Test mice are initially introduced to the central chamber and allowed to explore the entire apparatus for 10 minutes. The three-chamber social interaction test is designed to evaluate a mouse's sociability by presenting it with a choice between interacting with another mouse or an inanimate object. It is well-established that mice naturally prefer exploring a novel conspecific (another mouse) over a novel object.

Previous research has revealed that hTDP-43 mice exhibit a reduced exploration time of the social stimulus (social side) compared to control wild-type mice. [33] However, the time spent exploring the nonsocial stimulus between the two groups was the same. Notably, the total exploration time on the social side did not show significant differences. This observation suggests that reduced social interaction time may be caused by significant deficits in sociability rather than perceiving the stimulus as aversive, increased anxiety-like behavior, or decreased exploratory drive.

3.4 LANGUAGE TEST

While rodent models offer a valuable means to study early pathological changes, examining language in ALS or FTD in these models presents a

considerable challenge. The mutant tau mouse serves as one of the various mouse models for FTD. Menuet et al[65] investigated aged mutant tau mice and discovered impaired ultrasonic vocalizations correlated with tau pathology in the midbrain and brainstem nuclei responsible for vocalization and respiration. These findings may offer insight into the language disorders observed in FTD patients. Using ultrasonic vocalization as an indicator of changes in 'language' formation, mouse models can contribute to unraveling underlying mechanisms of semantic deficits in FTD. However, it is unlikely that a single species can fully capture the complexity of human language. Therefore, exploring species with more intricate vocalization patterns, such as songbirds and chimpanzees, may be a valuable avenue for elucidating the language-related features of FTD.

4. Sensory Capacity

It has long been believed that sensory neurons localized in the dorsal root ganglia (DRG) are not involved in ALS, and sensory neuropathy has not been widely recognized as part of the ALS syndrome. [66] However, accumulating evidence has revealed abnormalities in sensory neurons in both ALS patients and mouse models. A previous study found that Ia/II proprioceptive sensory neurons were injured by ALS-causing mutations, including $SOD1^{G93A}$ and $TDP\text{-}43^{A315T}$, in the pre-symptomatic stage of the disease. [67] A parallel study also shows that transgenic hSOD1(G93A) mice have both motor and sensory neuropathies, exhibiting mitochondrial damage to sensory neurons localized in DRG. [68] Nevertheless, while clinical and neurophysiological tests, such as thermal testing, [69] EMG, [70] and somatosensory-evoked potential assays, [71] have identified sensory nerve disturbances in individuals with ALS, there remains a significant gap in our understanding of sensory behavioral changes in mouse disease models. [72] It is widely recognized that sensory inputs can influence motor neuron activity and vice versa. Consequently, gaining insights into sensory behaviors in rodent models may shed light on certain ALS pathology.

5. Other paradigms

In addition to evaluating motor function, cognition and sentiment, and sensory capacity, researchers also employ a variety of other metrics when studying ALS. For example, weight loss serves as a reliable indicator of clinical deterioration in ALS. This observation can be made with minimal equipment and often represents the first sign of disease onset. In ALS rodent models, body weight loss is a common occurrence. However, due to the multitude of factors that can contribute to such weight loss, including reduced mobility and cognitive impairment, this phenotype is rarely utilized as a primary outcome in assessing ALS therapies.

Researchers have also employed the Phenotypic Neurological Scoring (NS) System to quantitatively assess the progression of ALS on a scale ranging from 0 to 4. [73] A score of NS-0 was assigned when mice displayed a normal gait during walking and exhibited normal splay when suspended by the tail. NS-1 marked the emergence of initial symptoms and was given when the gait remained normal, but signs of abnormal splay were observed. NS-2, indicating the onset of the disease, was assigned when hind limbs partially or completely collapsed during tail suspension and the toes curled downward. When a mouse reached NS-3, it displayed rigid hind limb paralysis during tail suspension, and the hind limbs were no longer used for forward motion. The humane endpoint, denoted as NS-4, was reached when no forward motion was observed due to extensive hind limb paralysis and progressive weakness in the upper extremities. Based on the description, this system is exceptionally objective when compared to other methods. However, when used in conjunction with the Kaplan-Meier survival test, it enables the observation of disease onset and humane endpoints in transgenic hSOD1(G93A) mice without the need for additional tools. [27,28] Moreover, it has the potential to avoid the influence of external stimuli induced by other behavioral tests used for evaluating ALS-related defects in rodent models.

6. Conclusion

To date, ALS remains an incurable disease with an elusive mechanism. The absence of a cure for ALS, along with the limitations of current therapies, highlights the intricate nature of this devastating condition. ALS is not limited to motor neuron dysfunction alone; it resembles a multifaceted and multisystem disorder where interneurons, DRG sensory neurons, and other systems contribute synergistically to disease initiation and progression. Due to this complexity, a single test may be insufficient to accurately quantify behavioral defects. Therefore, attention is needed to devise strategies for combining these tests to draw a reliable conclusion. Furthermore, given the variability observed in behavioral tests conducted on ALS rodent models, incorporating alternative species models into related assessments may contribute to a more reliable phenotype evaluation.

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