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Chapter 9

Neurobehavioral Testing in Prion Disease Studies

Davis M. Seelig , Michael A. Benneyworth and Damani N. Bryant

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Abstract

The prion diseases are neurodegenerative diseases characterized by progressive neurocognitive decline and terminal dementia. In this review, we will discuss the role of neurobehavioral testing in mammalian prion disease model systems, including (1) a review of the clinical phenotype of the major prion diseases in natural disease, (2) an evidence-based summary of the benefits and shortcomings of commonly used behavioral assays, and (3) a review of the neurobehavioral testing in rodent prion models. Based upon this review, and in light of the established importance of model systems in studies of prion pathogenesis and the proven role of behavioral testing in nonprion disease neurodegenerative diseases, it is vital that prion researchers consider the clinical consequences of prion infection so as to maximize the impact of their work.

Keywords: prion diseases, clinical signs, mouse models, behavioral testing, comparative neurosciences

1. Introduction

The prion diseases, or transmissible spongiform encephalopathies (TSEs), are a group of fatal neurodegenerative disorders resulting from the accumulation of a unique, nucleic-acid free/protein-only, infectious agent. Prion diseases affect both humans and nonhumans alike and include diseases that have genetic (familial or sporadic) or infectious causes. The pivotal and unifying event in prion pathogenesis is the posttranslational misfolding of the host-encoded, normal cellular prion protein (denoted PrP^{C}) into a misfolded variant (denoted PrP^{Sc} or PrP^{D}). Misfolding is characterized by increased β-sheet content, decreased α-helical content, and by conferred resistance to detergents, alcohol, formalin, proteases, boiling, autoclaving, and radiation [1]. The resulting PrP^{Sc} acts as a template for its self-propagation. In addition to their shared mechanism, prion diseases are united by their pathology, which includes amyloid deposition, vacuolization, synaptic dysfunction, glial-mediated neuroinflammation, and neuronal death.

Although the terminal pathologic event in prion disease is neuronal death and the terminal clinical event is neuronal death, the link between these is unclear. Historically, two competing hypotheses have been proposed, namely (1) a loss-of-function hypothesis or (2) a gain-of-function hypothesis. Based upon studies demonstrating that pre- and postnatal knockdown of PrP^{C} expression fails to replicate bona fide prion disease, it seems unlikely loss of function contributes significantly to prion pathogenesis [2–4]. However, while it is increasingly likely that an alternate isoform of PrP^{C} is responsible for prion toxicity, it is unclear whether this species presents a protease-sensitive or resistant form, a monomeric or oligomeric form, or if interactions with additional components are necessary. Lastly, while this model implicates PrP^{Sc} as a *necessary* player in the development of prion disease neurodegeneration, there is extensive work implicating that it is unlikely to be singularly *sufficient* to cause clinical prion disease. To this point, there are numerous studies demonstrating subclinical prion disease in which models accumulate often extensive amounts of PrP^{Sc} without developing clinical disease [5–10].

In a disease system rife with novelty, one of the most intriguing and clinically relevant aspects of prion disease biology is the existence of strains. Originally recognized in studies of sheep and goats with experimental scrapie, but the best characterized in scrapie-infected mice, the concept of strains reflects clinical, pathologic, and structural variants of prion disease [11]. Prion strains are unique isolates that demonstrate different phenotypical and biochemical differences when transmitted into identical hosts. Classically, these differences include pattern of PrP^{Sc} distribution (both within and outside of the CNS), PrP^{Sc} plaque morphology, vacuolar profile, incubation period, susceptibility to PK digestion, glycosylation profile, incubation period, and, most important for this article, clinical disease phenotype [12–14]. The biologic basis for strains is not entirely clear, but it is hypothesized that unique PrP^{Sc} confirmations and polymorphisms are significant contributors [15–17].

In a review of neurobehavioral testing in prion diseases, it is worth noting that there is not always a clear or proportional relationship between disease neuropathology (i.e., PrP^{Sc} accumulation, gliosis, and neuronal loss) and clinical phenotype. This is most dramatically represented in subclinical prion disease (i.e., measurable CNS PrP^{Sc} without clinical disease) and in prion-infected animals demonstrating significant clinical disease but lacking detectable PrP^{Sc} [7, 18, 19]. This lack of correlation between patterns of brain PrP^{Sc} deposition and clinical disease is well documented in many natural and experimentally infected TSE affected animals, including TSE-infected cattle, goats, and mice [18, 20–22]. In addition, a discordant relationship between neuronal loss and clinical signs is reported in BSE-infected cattle and between neuroinflammation and clinical signs in scrapie-infected sheep [23–27]. The cause(s) of this disparate relationship between PrP^{Sc} and prion disease are not completely clear, but the limited sensitivity of traditional PrP^{Sc} detection tools, the increasing recognition of the toxicity of protease-sensitive forms of misfolded PrP, and the complexity of the tissue response to misfolded prion protein likely contribute [27]. Finally, it is likely that shortcomings in behavioral testing have contributed to historical inabilities to document clinical disease in prion-infected animals, particularly those in which neurobehavioral deficits may be subtle. This is particularly likely in large animals, in which the vague and imprecise early clinical signs of TSE infection can mimic a number of nonprion infectious conditions.

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2. Clinical phenotype of prion diseases

Despite their unifying cause, individual prion diseases demonstrate unique clinical presentations. This clinical heterogeneity not only applies between differing diseases (i.e., CJD vs. FFI) but also within a particular disease. The following section summarizes the major clinical features of the most common prion diseases of humans and domestic animals.

2.1. Creutzfeld-Jakob disease (CJD)

Creutzfeld-Jakob disease (CJD) is the most common form of human prion disease and can be divided into sporadic, hereditary (i.e., familial), iatrogenic, or variant forms. The hereditary form can be further subdivided into three distinct phenotypic subtypes, namely (1) Gerstmann-Straussler-Scheinker (GSS) disease, (2) fatal familial insomnia (FFI), and familial CJD (fCJD). Although the following section will review the unique clinical features of each of these forms, all variants of CJD are generally characterized by a rapid, progressive onset of dementia of unknown origin [28].

Sporadic CJD (sCJD) is the most common form of CJD, representing approximately 85% of cases [29]. Although six major variants of sCJD are recognized according to differences in molecular, genetic, and biochemical features, most CJD variants present a similar clinical phenotype [30, 31]. The common features of CJD are represented by progressive dementia with some combination of myoclonus, visual deficits, cerebellar disturbances, pyramidal or extrapyramidal symptoms (spasticity, hyperactive reflexes, muscle contractions, alterations in movement, tremor) or akinetic mutism (alertness with a lack of motor functions, including speech, gestures, and facial expression) [32]. However, notable clinically unique CJD subtypes include cerebellar (or ataxic subtypes), myoclonic CJD, thalamic CJD, and the Heidenhain variant (which manifests significant visual deficits) [33–36]. In addition to these variants, 41 distinct forms of inherited TSEs have been described in humans, each demonstrates unique clinical phenotypes unique point mutations or octapeptide insertion mutations [32].

Fatal familial insomnia (FFI) is a clinicopathologic variant of human prion disease considered to be a familiar variant of CJD. Genetically, FFI is characterized by a mutation at codon 178 of the prion protein gene (aspartic acid to asparagine) coupled to a methionine polymorphism at codon 129 on the corresponding abnormal allele. As the name indicates, FFI patients chiefly suffer from sleep disturbances—principally insomnia, but also including hypersomnia, restless sleep, and sleep attacks [37]. Beyond these, FFI patients demonstrate a range of clinical signs that are both similar to, and unique from classic CJD. Overlapping signs include cognitive deficits, spatial disorientation, ataxia, and hallucinations whereas clinical signs unique to FFI include weight loss, hyperhidrosis, and husky voice [38]. However, even among FFI patients, there are unique clinical syndromes that depend upon the codon 129 genotype. For example, it has been reported that hallucinations and myoclonus are more common in patients that are methionine homozygous (i.e., MM) at codon 129, whereas vegetative disturbances and nystagmus are more common in methionine heterozygous patients [37]. Interestingly, although the diagnosis of unique variants of prion disease based on clinical phenotype only is considered

difficult, an algorithm of FFI specific and sensitive clinical signs has been developed which correctly identified 81% of patients during early disease stages [38].

Like FFI, Gerstmann-Straussler-Scheinker (GSS) is a mutational variant of CJD in which a number of differing prion protein gene point mutations have been identified, the most common of which is the P102L/129M variant [29]. There are two typical clinical phenotypes of P102L GSS, namely (1) a typical type with cerebellar ataxia and slow onset dementia and (2) a CJD-like form with acute dementia and myoclonus [29, 39].

2.2. Scrapie

Like other TSEs, scrapie is a clinically progressive disease that is most classically characterized by pruritus, altered behavior, and locomotion deficits [40]. However, like other prion diseases, the clinical phenotype of sheep scrapie varies somewhat according to strain and host characteristics. Accordingly, three profiles of clinical disease have been described, namely (1) a pruritic form, (2) a paralytic form (which lack pruritus), and (3) an atypical cerebellar (Nor98) form [41]. The neurologic signs of scrapie are wide-ranging, and include mentation abnormalities (e.g., hyperresponsiveness), motor deficits (e.g., incoordination, exaggerated gait, hypermetria, ataxia, tremors), visual deficits (including nystagmus and blindness), loss of the menace response, dysphagia, and dysphonia [42, 43]. Although not always the case, deficits in locomotion, including hypotonia, proprioceptive deficits, reduced withdrawal reflex, and ataxia, are reported to occur later in disease [27, 43]. Terminal sheep scrapie is characterized by depression, recumbency, and/or seizure activity. In addition to the classical form of the disease, an alternate strain of scrapie, denoted atypical or Nor98, has been described and is characterized clinically by motor deficits, including progressive ataxia and incoordination whereas pruritus is very uncommon [44]. Scrapie-infected goats demonstrate many of the same clinical signs as seen in sheep, including pruritus, restlessness, and terminal ataxia/recumbency [21]. Similar to sheep, discrete clinical phenotypes have been identified in goat scrapie, namely a "scratching syndrome" characterized principally by pruritus and a "drowsy syndrome" characterized by decreased activity and depression absent pruritus [21, 45, 46]. However additional features have been reported, including teeth grinding, irritability, and heightened alertness [42]. Additional noted differences between scrapie-infected sheep and goats include hyperesthesia in goats (as opposed to hypoesthesia in sheep) and nibbling of the body in goats (as opposed to rubbing of the body in sheep) [21].

2.3. Bovine spongiform encephalopathy (BSE)

In contrast to the prion diseases of nondomestic species, the clinical features of BSE-infected cattle are well described. Like other prion diseases, BSE infection in cattle is principally associated with progressive changes in behavior and locomotion. Early disease is dominated by changes in behavior, including increased alertness, nervousness, excitability, nervous ear/eye movements, and hypersensitivity to touch, sound, and visual stimuli, head shyness, panic-stricken response, reluctance to enter the milking parlor, and change in temperament [20, 47–49]. During this early phase, specific tests used to elicit hyperesthesia include: (1) the

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"flash test" (reactivity to a camera flash), (2) the "clipboard test" (reactivity to waving a clipboard towards the animal, (3) the "hand clap" (reactivity to clapping hands), and (4) the "stick test" (reactivity to a light touch of the hindlimbs with a flexible stick) [50]. As disease progresses, BSE-infected cattle develop deficits in locomotion include tremors, hypermetria, hindlimb and generalized ataxia, difficulty rising, spastic gait, and thermal recumbency [49]. Terminally, cattle may enter into a "dull" form of the disease characterized by loss of previous hyperesthesia and disinterest in surroundings [20]. Previous studies have shown that at least one, either apprehension, hyper-reactivity, or ataxia, is found in 97% of cattle with BSE [51].

Outside of cattle, there is sparse information on BSE infection in other species. In BSE-infected goats, hyperesthesia, pruritus, head tossing, or shaking, overreactivity to touch of the hindlimbs, and hypermetria are reported [21]. There are conflicting reports on the clinical phenotype of BSE-infected sheep, which may reflect route of inoculation, age of infected sheep, or intensity of clinical monitoring. In one report, BSE-infected sheep demonstrate a uniform clinical disease characterized by early pruritus with late locomotion deficits [41]. Whereas, other studies suggest that sudden-onset ataxia is common in BSE-infected sheep [52].

In addition to classical BSE (C-BSE), two unique strains of BSE have been described. These strains denoted by H-BSE and L-BSE according to their biochemical characteristics and migration profile of the proteinase-resistant fragments on Western blot, demonstrate some clinical features unique from C-BSE. Similar to C-BSE, cattle experimentally infected with either H-BSE or L-BSE demonstrate both hyperesthesia and dullness, however the magnitude of hyperresponsiveness is reported to be higher in C-BSE [20]. While no consistent differences were noted when the clinical phenotype of H- and L-type BSE were compared, cattle with either of these two forms of atypical BSE did not progress to permanent recumbency and failed to demonstrate tremors, which contrasts with C-BSE [20].

2.4. Chronic wasting disease (CWD)

Chronic wasting disease (CWD) is an endemic prion disease of cervids, affecting white-tailed deer, mule deer, elk, moose, red deer, sika deer, muntjac, and reindeer. The two most recognized clinical signs of natural CWD are behavioral changes and loss of body mass. Not surprisingly, the behavioral phenotype of CWD in wild, naturally infected animals is not well-described, but work with captive (both naturally and experimentally infected) animals has provided some descriptive insights. Like other ruminant TSEs, CWD is a progressive disease. Early in the progression of CWD, the behavioral abnormalities in CWD are considered subtle and best appreciated by those who are in repeated contact with infected animals. Early clinical signs include alterations in patterns of interaction with humans (either increased or decreased contact), fixed gaze, repetitive behaviors (head tossing, exaggerated lifting of the legs), diminished alertness, prolonged periods of somnolence, and aggressive behavior which, late in disease, progresses to motor deficits (incoordination, trembling, and stumbling) [42, 53, 54]. Although distinct strains of CWD have been identified, as reflected by incubation period and neuropathologic differences, their neurobehavioral characteristics have not been reported [55, 56].

3. The basic toolkit of behavioral phenotyping

Behavioral research in laboratory rodent species has progressed for decades, largely with the aim of understanding the biological basis of normal behavior and brain function. When properly utilized, behavioral analysis has the potential to be both explanatory of the *in vivo* impact of underlying molecular changes and by suggesting novel areas of dysfunction. With the advent of gene targeting, focus has begun to shift toward the utility of behavioral analysis within the context of disease modeling and drug development. This disease focused behavioral research can be looked at assays as falling into three gross categories [57]; behavioral models of a disease state (e.g., self-administration of cocaine by rodents as an addiction model), behavioral bioassays of specific neural activity (e.g., stereotyped head twitch responses to drugs targeting serotonin 2A subtype receptors), or screening tools to assess the impact of biological manipulations (chemical/pharmacological, genetic, or neurological). It is in this last category that most of the present discussion falls where we will look at some of the tools that are widely used in behavioral phenotyping analysis. For simplicity, the tools are broken down into three broad categories of behavior: neuromotor function, learning and memory, and anxiety and depression-related behavior.

3.1. Neuromotor function

One of the first classes of behaviors that is often looked at in phenotyping studies, is the effect of the manipulation on neuromotor function, e.g., general activity, coordination, strength. A wide array of assays is available to assess the diverse aspects of neuromotor function. All of these assays are very approachable and several are amenable to automated scoring systems (for further review see Pierce and Kalivas and Wahlsten) [58, 59]. The main differences to note in the assessment of these tests are the aspect of motor function being examined, the context of the test environment, and the motivational drive for movement.

Open field locomotion test. Animals are placed in a novel, open test arena and distance traveled is determined for anywhere from 10 to 120 min, depending on the goals of the testing. The test arena can be almost any shape, but square is most common. Automated scoring is achieved through either beam breaks of a photocell grid or by video-based tracking of animal position. Exploration of the open field is driven by the novelty of the test arena. As such, with additional time (or repeated exposures) activity levels decline. Repeated testing can be used to assess habituation learning. Data in this test is generally binned to look at changes in activity over time, or presented as a single measurement of distance traveled during the test.

Home cage running wheel activity. While open field locomotion provides a rapid way to assess general activity, it does present a limitation by measuring activity in a foreign environment. So, activity level can be confounded by anxiety/stress responses in unexpected ways. Measurement of activity in the home cage overcomes this limitation, and additionally provides the opportunity to measure activity over long periods of time. Computer-tracked wheel running systems are used to count rotations. Critical to the use of these systems is the understanding that it can take several days for a mouse to figure out the running wheel, and begin high rates of running. It is also noteworthy that activity follows a robust circadian pattern, with running

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activity ramping up during the dark-phase. With studies of home-cage wheel running, investigations can simply look at the magnitude of activity, degree of entrainment to light cycle, or alterations in the free-running cycle observed in the absence of external light cycle. In addition to running wheels, photocell grids can be placed around the home-cage to measure horizontal movement. This affords the ability to measure normal home cage ambulation, but it is unclear if such studies display the same robust circadian rhythms in activity as mice may spend much of their active time digging and grooming as opposed to ambulating. An unavoidable source of confound in these home-cage activity studies is the need to single-house the mice which can have dramatic, if not variable, effects on behavior.

Rotarod. More a test of coordination and balance than general activity, the rotarod assesses the ability to walk on a continually (often accelerating) rotating rod, where the aversion to falling motivates the mice to keep walking. Animals are placed on the rotating rod and the latency to fall is determined in multiple trials across 3–4 days of testing. The repeated testing days gives an assessment of motor learning that is not easily achieved by other measures. The accelerating rotarod protocol is a task fairly sensitive to motor impairments, as the increasing speed becomes a fairly difficult task for mice, and is well suited to longitudinal studies. The confounders in this test are few, but two behaviors can emerge that can affect the validity of the data: (1) mice decide that falling is not aversive and (2) mice develop the ability to grasp onto the rod and rotate instead of walking on the rod. Both of these confounding behaviors present the investigator with a decision of whether to exclude data or, in the case of the later situation, manually stop a trial. With logically applied criterion, these confounds can be minimized and the task can retain its high sensitivity to motor deficits.

Balance beam test. The balance beam test simply consists of training mice to walk across a balance beam, from a brightly lit start position to a dark enclosure at the end of the beam [60]. Training takes 2 or 3 days, then the mice are tested on beams of differing diameters (10–25 mm) and shapes (square vs. round). The basic data measure is latency to cross the beam and the number of hindpaw slips that are observed. Both time and footslips are sensitive to subtle impairments. The apparatus for this test is easy to construct and scoring is done by a trained observer, making this a fairly easy assay to set up in any lab. Additionally, we have found this assay to be useful in longitudinal test designs, as the mice retain the initial training and do not often need as much follow-up training.

Gait analysis. Gait analysis can be performed in mice using paw-inking methods or through the use of more sophisticated video-based paw tracking software. The latter method employs a high speed camera mounted below a transparent walkway or treadmill and computer-assisted tracking of individual paws. The software for these systems is capable of tracking numerous metrics about stride characteristics (swing, breaking, propulsion), as well as providing information about stance width and paw placement angles. Though quite useful in terms of the variety of information, these systems can be expensive, require significant user review of the paw tracking analysis, and significant amount of research into the various domains in the gait analysis to understand their utility.

Grip strength. Various apparatus have been developed to assess grip (muscle) strength in mice. Very simple tests using inverted screens or wire can be used to assess hanging duration, or the

ability to hang on to objects of varying weight can be timed [61]. These timing-based measures are very simple to employ but may not offer the sensitivity or accuracy of more sophisticated tools using force sensors to measure the strength of an animal to hold onto a grid or rod (in response to an opposing force applied by the experimenter). These metric are largely devoid of the motivational confound in other tests and provide complementary information.

3.2. Learning and memory

Another broad category of behavior that is regularly looked at is learning and memory (cognitive function). Assessing cognitive function can take many forms as there are multiple domains of cognitive function. Some of the basic domains include spatial navigation learning, working memory, and conditioning can be readily studied in mouse models without complicated and prolonged training. Additionally, each of these tests measures very different functions that involve different neural circuitry.

Spontaneous alternation tasks. For measuring spatial working memory via spontaneous alternation task, one of two variants (T- or Y-maze) can be used. The T-maze task is a very simple way to assess working memory function, utilizing a T-shaped maze that consists of a start box and two choice arms. This task is based upon optimizing foraging strategies suggesting that the animal will alternate entries into choice arms, so as to avoid arm previously explored. Animals will typically display ~70% spontaneous alternation. The use of a start box in T-maze task allows for discrete trials and control of intertrial intervals. Varying the intertrial interval can modulate the working memory load on the mice and alter the "difficulty" of the task. Rewarded versions of this task are often utilized that would allow an investigator to drive performance above 85% alternation, providing higher detection window for deficits. Also, the rewarded version can be utilized for repeated testing to observe the effects of manipulations during testing in the same mice. A continuous performance version of this task, the Y-maze, is often used and presents an animal with a radially symmetrical maze where all arms are in effect choice and start positions, which offers an investigator an opportunity to observe exploration continuously without external interruptions.

Spatial navigation tasks: Morris Water Maze (MWM) and Barnes Maze (BM). These are widely employed tests of hippocampal-based spatial navigation learning. The MWM involves placing the test subjects in a large (<1 m diameter) water tank, where the subjects must find the escape platform that is hidden just beneath the water surface. Using distal, extra-maze visual cues that remain in a fixed position relative to the escape platform, acquisition of the task can take anywhere from 4 to 10 days. Subsequently spatial memory is assessed in a "probe trial," during which time there is no escape platform and the memory for the platform position is determined. The major metrics of memory include, exploration bias (typically a quadrant analysis of exploration of the tank), average proximity to or number of crosses of the platform location, or latency to first approach the platform location. Analysis is effectively performed by commercially available video-based tracking software. While this test has become widely adopted, it is not without confounds, notably confounding swim strategies such as floating and thigmotaxis. Often a response to the stress of the test, these behaviors can complicate the use of any of the time-dependent (including exploration bias) measures.

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Additionally, this test can be very sensitive to, and negatively impacted by, motor impairments and/or sensitivity to effects of water exposure on body temperature. The Barnes Maze is a dry-land version of the water maze, originally developed for rats as a way to avoid some of the motivational confounds of other tests that utilized strongly aversive stimuli. Subsequent studies adapted the procedure for mice [62]. The BM involves an elevated circular platform, with numerous (e.g., 20) holes located on the perimeter of the apparatus, one of these holes leads to an escape box. The maze is lit from above and the combination of the light and openness serve as a motivator to encourage mice to escape from the maze to a small, dark enclosure, to be then returned to their home cage. Just as in the MWM, extra-maze cues are used to navigate to the escape hole and software can be used to analyze behavior. In addition to the mentioned advantages over MWM, the lack of a water tank and the use of a collapsible test platform make the BM a great choice for space constrained investigators/facilities in need of tests that confer a high degree of modularity to a test space with a minimal amount of setup, breakdown, and cleanup.

Fear learning. Fear learning tasks involve the assessment of a behavioral response to cues associated with an electric shock. In avoidance tasks (e.g., two-way active avoidance) the subject learns to shuttle between the two sides of a chamber in response to predictive cues (tones or lights), as such an avoidance response prevents or terminates the shock. Retention of the conditioning in tested in a subsequent test session. This procedure actually involves two interacting forms of learning, classical, and operant conditioning [63]. The classical conditioning involves association of the predictive cue (conditioned stimulus) and the shock (unconditioned stimulus), leading to the enhancement of an innate fear response. Subsequently, operant conditioning occurs whereby the animal develops an escape response as it learns that this operant response leads to termination or avoidance of the shock. As a result the neural circuitry involved in this test is more complicated than behavioral tests where the only classical or operant conditioning is utilized. To some degree the conditioned fear (often resulting in freezing) is also at odds with the avoidance behavior, so clear interpretations of slower escape latencies can be unclear if the mice are quick to display a freezing response. As an alternative to avoidance tasks, conditioned freezing procedures that exclusively utilize classical (Pavlovian) conditioning can be utilized. Multiple procedural variants have been developed that present an inescapable shock in combination (or not) with discrete cues. The animals adopt a freezing response to both the context in which the testing takes place, as well as to the cues. In one of the most widely used variants, delay fear conditioning, the brief footshock is presented in cotermination with the cue (sounds and/or light), and the predictive association that forms between the discrete cues and the shocks can drive a long-lasting fear response. The fear that develops to the cues is suggested to involve neural processes involving the amygdala, while information about the test environment (context) also takes on fear-inducing qualities related to hippocampal function [64]. Variants of this task can be employed to selectively look at contextual fear (no paired cues) or time intervals can be utilized in between the termination of the cue and the presentation of the shock in so-called trace fear conditioning tests [65]. This variation of adding the trace interval alters the circuitry involved in memory formation so involve a more complex circuitry that involves the hippocampus and prefrontal cortex [66].

3.3. Anxiety and depression-related behavior

This is an area of research typified by some very approachable tests that are useful in their own right to study the impact of manipulations on anxiety and depression-related behavior [67, 68]. These assays are also important tools to use as controls for altered motivation in cognitive assays [69]. These assays are often fairly easy to employ, but can be easily impacted by uncontrolled external variabilities, and many times subject to misinterpretation/overinterpretation of data. Critical to effectively using these behavioral tests is an understanding their test validity, be it construct, face, or predictive [70]. Also, as there is some inherent fallibility in interpreting these behaviors as they relate to affective and mood disorders, it is important to utilize multiple tests in combination for a thorough evaluation.

Exploratory conflict tests: elevated plus maze (EPM) and the open field test (OFT). The EPM test offers the animal an opportunity to explore two distinct zones, closed arms and open arms, of a plus-shaped maze. The open arms are the more aversive environment as they are more brightly lit than the closed arms and do not contain the side wall enclosure. Aversion to the open arms can be modulated, to a degree, by altering the open arm light levels. Additionally the test platform is elevated (~1 m) to enhance the aversive nature of the open arm and deter a possible escape route (i.e., jumping off the maze). In assessing exploration, the preferred metric is to look at open arm time as a function of total arm exploration time. This measure avoids confounds of interpreting data collected while the mouse is in the center zone of the maze, where indecisive exploration of the arm openings is apparent. Data in this task is fairly resistant to hyper/hypoactivity confounds and has been shown on numerous occasions to be responsive to proven anxiolytic drugs (i.e., predictive validity) [71, 72]. The OFT is simpler to run, though perhaps the more difficult to interpret. The OFT looks for anxiety by assessing the pattern of exploration of a novel arena. In this assay, changes in the exploration of the center zone (e.g., 40 × 40 cm) of a large arena (e.g., 50 × 50 cm) examined as a measure of anxiety. This test is conceptually similar to the EPM test in the exploration conflict and attempting to quantify the same aversion to open spaces. However, this test can easily show false positives (e.g., psychomotor stimulants) and in our hands has not consistently shown to be sensitive to benzodiazepine anxiolytics.

Stress-induced hyperthermia assay (SIH) and social interaction test. Two nonexploration-based tasks that are sensitive to changes in anxiety are the SIH assay and social interaction test. SIH measures the physiological response to stress (increase in body temperature) that is shared across warm-blooded animals [73]. This measure is particularly effective at identifying anxiolysis, and as such is a good screening tool for novel anxiolytic drugs. The social interaction test is an observer scored assay that scores the interaction of two freely moving mice in a novel test environment. Elevations in anxiety levels of a test subject are thought to be reflected as a decrease in affiliative responses (grooming, sniffing, etc.) to a novel social partner. Beyond anxiety, this assay is also utilized in the neuropsychiatry literature in models of diseases where social deficits are present, e.g., autism and schizophrenia.

Forced swim test (FST) and the tail suspension test (TST). FST and TST are tests of stress-coping responses. These tests look at the behavioral response of subjects to an inescapable stressor. FST puts mice in an inescapable water tank, while in the TST mice are inverted and suspended

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by their tails. Both tests are fairly brief (5–6 min) and look to quantify the level of immobility, viewed as the adaptive response that develops during the test. Automated analysis of these behaviors has proven quite effective for scoring large numbers of test subjects. In some variants of the FST, investigators will use long exposures to swim stress prior to the actual testing, in order to precipitate a stronger immobility response.

Two-bottle sucrose preference test (SPT). Anhedonia is specifically a symptom of depression which is characterized by a lack of pleasure seeking. In rodents, there are multiple ways to assess this, but the most readily utilized measure is the SPT, which compares consumption of a sucrose solution to normal water in a home cage setting over a several day period, with increasing sucrose concentrations resulting in an increased preference. Anhedonia is observed as a reduction in preferred consumption of sucrose as compared to water.

This discussion of behavioral testing has mostly focused on individual tests, what they are, how they work, what is the utility and what the confounds are to their use. However, at this point it is important to discuss the use of combinations of tests into so-called "test batteries." The idea of a broad-based analysis of behavior is at the heart of behavioral phenotyping efforts that have grown in response to advances in murine genetics and increasing emphasis on disease modeling research (for review see Crawley) [74, 75]. The construction of a proper test battery is not a trivial or even standardized operation. Test batteries can be designed to be intentionally broad with an emphasis on observation and characterization as is often done with gene knockout studies. Such designs tend to take a relatively agnostic approach to hypotheses about phenotype and may use an initial screen to suggest more detailed behavioral analysis or follow-up mechanistic studies. Another way to design a screen is with investigation of a very specific endpoint in mind (e.g., cognitive deficit). In this case supplemental tests may be chosen to satisfy controls for confounding behavioral deficits (motor dysfunction, sensory deficit, or changes in motivation). In all situations it is advisable to at least consider the use of multiple tests within the same behavioral domain that utilize different outputs or behavioral abilities to complete the test.

4. Behavior assays used in mouse models of prion disease

The adjective insidious is commonly used to describe the prion diseases because there are no obvious outward symptoms to alert the public to infection and progression. This presents a problem to those seeking to provide a therapeutic intervention. A common theme in medicine is the idea that early intervention in disease progression is more likely to lead to a better prognosis. Thus, the conundrum with prion diseases is that since this disease progresses silently, how are we to be alerted to its progression in order to intervene? Luckily for us, prion diseases are neurological diseases and there is an expansive literature on brain—behavior relationships. Thus, behavioral testing using experimental animal model systems allows for sufficient control of variables to rigorously test specific hypotheses about the impact of prion disease progression on behavior. As such, there have been a number of studies that have attempted to use behavior assays to document the progression of prion diseases.

Although this chapter focuses on the utility of behavior analysis for understanding prion diseases, it is interesting that early studies used scrapie to understand brain-behavior relationships. Savage and Field used the open field test to measure emotionality (at various dpi) in mice that were intracerebrally (IC) inoculated with scrapie (third passage from sheep) [76]. Their data indicate that disease progression is correlated with a decline in emotionality, but not ambulation. A subsequent study used 263 K scrapie inoculum to unilaterally ablate the striatum in golden hamsters [77]. Striatal destruction was verified using the apomorphine stimulated rotation task. The authors suggest that scrapie might be a useful tool for studying other brain regions such as the basal ganglia.

Clinical signs of disease progression in IC inoculated scrapie mouse model systems are observed around 23 weeks or 161 days post inoculation [78, 79]. By this time, the disease has progressed to the point where no therapeutic intervention will succeed. Mice at this stage of the disease show reduced mobility, hunched posture and lack of grooming [78, 79]. Heitzman and Corp wanted to determine if they could detect behavioral symptoms of scrapie prior to the then current standard of 16 weeks post inoculation [80]. They tested mice that were IC inoculated with scrapie using the open field test and the emergence test. Although they did not observe any effect of early disease progression on the open field test, they did observe a statistically significant effect of scrapie on the emergence test at 6 weeks post inoculation. This data suggests that scrapie inoculated mice show reduced exploratory behavior or increased anxiety. More importantly, this data also indicates that it is possible to observe changes in behavior 9 weeks prior to the onset of clinical symptoms in scrapie-inoculated mice.

Outram put forth several "to be met" criteria required for scrapie-behavior correlations [81]. (1) The behavior change must be a consequence of scrapie. (2) One should determine whether the change in behavior is correlated with altered central or peripheral nervous system activity. (3) The behavioral assay itself should not modify disease progression. (4) The behavior assayed and its neural bases should be well characterized. With these criteria in mind Outram demonstrated that drinking behavior is altered in IC inoculated scrapie mice [81]. Declines in drinking behavior were observed approximately 7 weeks post inoculation using a number of fluids, including sucrose, water, and glucose + saline. This finding was seen in mice that were IC or IP inoculated with several scrapie strains including ME7, 22A, 79A. This effect was also observed in several mouse strains, including C57BL, A2G, VL, and VM mice.

Subsequent work by McFarland et al. found that both mouse strain and scrapie strain affected the open field and Y maze performance [82]. In Nya:NYLAR, C57/10J, and ICR mice that were IC inoculated with Chandler scrapie, only ICR mice showed a statistically significant reduction in spontaneous alternation in the y-maze task. Moreover, Y-maze performance was diminished in the Nya:NYLAR and ICR mice, but not C57 mice. In the second experiment Nya:NYLAR mice were IC inoculated with one of three scrapie strains: 22C, ME7, and 79-A and tested at 95–103 dpi. The 22-C inoculated mice exhibited a statistically significant decrease in activity, but 79-A mice exhibited a statistically significant increase in activity. Moreover, only the ME-7 and 79-A strains resulted in a reduced entry into the center field. Although there was no effect of scrapie strain on y-maze spontaneous alternation, 79-A inoculated mice exhibited an increased number of arm entries. The strain specificity of prion clinical phenotype was further demonstrated by a study examining behavioral effects on C57BL/6 mice IC inoculated with

either the scrapie strains 139A or ME7 or the mouse adapted BSE strain 301C [83]. Mice inoculated with 301C were generally less active during the dark phase of the light-dark cycle than control or scrapie inoculated mice. In contrast, ME7 inoculated mice also showed a decline in activity during the dark phase, although not to the same extent as 301C inoculated mice. Statistically significant scrapie strain effects were observed in measures of the duration of several open field behaviors including, rearing, wall rearing, sniffing, grooming, and walking [83]. Scrapie inoculated mice did show a decline in water consumption around 10 weeks post inoculation, consistent with data published by Outram [81]. All mice exhibited similar scrapie induced neuropathological changes [83]. Taken together, these studies indicate that scrapie strain and mouse strain may impact the outcome of behavioral assays.

More recently, a battery of behavioral tests has been successfully used to visualize the progression of prion disease across several scrapie strains [61, 78, 79, 84–86]. Based on their work over the years, the aforementioned authors have elucidated the timing of behaviors that are affected. Nesting and affective behaviors (glucose consumption and burrowing) are first to be affected. Motor, strength, and coordination deficits appear subsequently. Finally, mice show decreased activity and prototypical clinical signs of scrapie. Betmouni et al. took advantage of evidence that the ME7 scrapie strain apparently targets the hippocampus, in order to determine if behavioral testing is useful for detecting early, subtle, hippocampal deficits in scrapie inoculated mice [78, 79]. Hippocampal deficits have been associated with hyperactivity and deficits at passive avoidance tasks. The authors observed increased locomotor activity and impaired retention of a multitrial passive avoidance task in scrapie inoculated mice around 12–14 weeks post inoculation. The authors also observed motor function impairments on the inverted screen and horizontal bar tests before the onset of known clinical signs of scrapie. A subsequent study examined the behavioral correlates of scrapie progression using a similar battery of tests [87]. Burrowing of food in the home cage was found to be inversely proportional to disease progression in scrapie inoculated mice. Consistent with other studies there was a decline in spontaneous alternation, beginning around 10 weeks post inoculation and there was a statistically significant reduction in glucose consumption in scrapie inoculated mice during weeks 15–19. A statistically significant effect of group was also observed in the horizontal bar test, which tests motor coordination [84]. The authors did not observe any statistically significant differences between groups in the rotarod or the inverted screen test. In sum, the development of a battery of behavioral assays is a boon for science in that it facilitates the comparison of experimental findings across investigators.

As previously discussed, early studies provide evidence that both scrapie and mouse strain may impact on the outcome of behavior assays. Cunningham et al. examined the behavioral progression of scrapie in C57BL/6J mice inoculated with one of the following strains: ME7, 79A, 22L, and 22A [86]. All mice were intrahippocampally inoculated with one of the aforementioned scrapie strains or normal brain homogenate. After recovery mice were subjected to the battery of behavioral tests described above. A similar disease progression was observed in all scrapie inoculated mice, except those that were inoculated with 22A. These mice exhibited a delayed disease progression. ME7 inoculated mice were the first to show decreased glucose consumption around 10 weeks post inoculation, followed by 79A and 22L at 12 weeks post inoculation. In these mice, although the progression of scrapie was generally similar, there were differences in end stage neuropathology. Although all scrapie inoculated

mice showed microglial activation, the degree of activation appeared to be less in the 22L inoculated mice. There were strain differences in vacuolation in the hippocampus, septum, and thalamus. Although all scrapie inoculated mice showed widespread PrP^{SC} staining, there were also strain-dependent differences in the density of scrapie with some strains showing more diffuse immunoreactivity and others show plaques or punctate immunoreactivity. Neuron loss was fairly similar in all scrapie inoculated mice. One striking finding was that there was a lack of hippocampal cell death in 22L or 22A inoculated mice, despite the fact that all scrapie inoculated mice received an intrahippocampal injection. The authors note that this is consistent with the idea that variables other than site of exposure contribute to PrP^{SC} spread and neuropathology.

Taken together this brief review of the literature indicates that it is possible to use behavioral testing as a proxy to monitor the progression of prion disease in mouse model systems. An important caveat, however, is that investigators must carefully consider scrapie strain effects, mouse strain effects or interactions between the two. Although this is an important variable to consider, there are exceptions to this generalization. For instance, Asuni et al. noted that their previous studies used C57BL/6J mice from Harlan laboratories, a mouse strain that was subsequently shown to have a spontaneous deletion of alpha synuclein [88]. The authors were concerned that the absence of alpha synuclein represented a potential confound with data that correlate synaptic loss with prion disease progression. A comparison of C57 mice with and without alpha synuclein revealed no impact of alpha synuclein on the progression of scrapie as assayed by behavioral testing.

4.1. Behavioral studies in transgenic mouse models

4.1.1. Behavior assays have been used to validate prion knockout mice

As mentioned earlier, our current understanding of prion disease is that it is a consequence of misfolded PrP^{C}. However, the function of PrP^{C} is not wholly known. To further understanding of its function, a number of groups have developed PrP^{C} knockout (PrP^{KO}) mice. As part of these studies, behavior assays have been used to assess the impact of PrP ablation. The first PrP^{KO} mouse, also known as the *Zurich 1* line was generated in 1992 [2]. This first KO mouse was highly anticipated and a number of behavioral tests were performed across several studies in order to elucidate the normal biological function of PrP^{C}. Surprisingly, Bueler et al. reported that the mice did not show any gross anatomical or immunological abnormalities [2]. These mice did not show any deficits in spatial navigation on the water maze test even after 2 years [89]. These mice also failed to show any deficits in the y-maze discrimination test, or the two-way active avoidance test. These data suggested that the mice did not have any deficits in hippocampal-dependent spatial learning and memory, problem solving strategy and hippocampal-dependent associative and nonassociative learning.

However, other researchers have found that *Zurich 1* mice do demonstrate behavioral deficits, including altered circadian locomotor behavior, increased number of crossing in an open field test, and a decreased in latency to step down (i.e., memory impairment) in reference [90–92]. The memory impairment of the PrP^{KO} mice appeared to be more prominent on long-term memory (24 h retention) than short term memory (90 min retention), though this difference is likely related to the poor memory retention of the control mice in the short-term memory test.

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Additionally, *Zurich 1* mice have been shown to have impaired swimming capacity, the magnitude of which increased as the task difficulty increased [93].

Meotti et al. used a number of thermal and chemical nociception tests, to determine whether PrP^C has a role in pain detection [94]. *Zurich 1* mice also show an increased latency to remove the tail during the tail flick test, an assay of thermal nociception and a transient increase in the number of abdominal constrictions in response to IP injection of acetic acid, which is a visceral nociception test [94]. *Zurich 1* mice also show olfactory deficits, as assayed by the buried food test [95]. Lastly, *Zurich 1* mice have been shown to have increased aggressive behavior relative to wild-type controls as measured by the resident intruder test, which measures aggression in males in response to novel intruder males [96, 97]. In addition to prion protein ablation, the impact of PrP^C overexpression has been examined. Lobao-Soares et al. examined a number of behaviors including locomotor, exploration, and anxiety using the rotarod, open field and elevated plus maze, respectively, in PrP^C overexpressing mice [98]. Their data indicate that PrP^C overexpression was associated with better performance on all tasks [98].

5. Future directions

This review of the behavioral effects of prion disease has attempted to demonstrate the dramatic, host, agent, and disease-specific heterogeneity in natural and experimental systems. While these differences are recognized, the reasons underlying them are not known. As much as this unknown reflects uncertainties regarding the mechanisms of prion neurotoxicity, it also demonstrates the limited body of work that has systematically cataloged and characterized the clinical deficits these systems. Due to this knowledge gap, in concert with a growing understanding of the scientific importance of behavioral testing, it is important that prion researchers continue to consider clinical phenotype in future *in vivo* prion investigations.

Author details

Davis M. Seelig[1]*, Michael A. Benneyworth[2] and Damani N. Bryant[1]

*Address all correspondence to: dseelig@umn.edu

1 Veterinary Clinical Sciences Department, University of Minnesota, Saint Paul, MN, USA

2 Department of Neuroscience, University of Minnesota, Minneapolis, MN, USA

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