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Behavioral phenotyping of mouse models of Parkinson's Disease

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Abstract

Parkinson's disease (PD) is a common neurodegenerative movement disorder afflicting millions of people in the United States. The advent of transgenic technologies has contributed to the development of several new mouse models, many of which recapitulate some aspects of the disease; however, no model has been demonstrated to faithfully reproduce the full constellation of symptoms seen in human PD. This may be due in part to the narrow focus on the dopamine-mediated motor deficits. As current research continues to unmask PD as a multi-system disorder, animal models should similarly evolve to include the non-motor features of the disease. This requires that typically cited behavioral test batteries be expanded. The major non-motor symptoms observed in PD patients include hyposmia, sleep disturbances, gastrointestinal dysfunction, autonomic dysfunction, anxiety, depression, and cognitive decline. Mouse behavioral tests exist for all of these symptoms and while some models have begun to be reassessed for the prevalence of this broader behavioral phenotype, the majority has not. Moreover, all behavioral paradigms should be tested for their responsiveness to L-DOPA so these data can be compared to patient response and help elucidate which symptoms are likely not dopamine-mediated. Here, we suggest an extensive, yet feasible, battery of behavioral tests for mouse models of PD aimed to better assess both non-motor and motor deficits associated with the disease.

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease and is widely thought to primarily affect the dopamine (DA) neurons of the substantia nigra pars compacta (SNpc). PD is considered to be due to the combination of genetic and environmental factors [1-3]. Most animal models of the disease have stemmed from this concept, and have employed a myriad of genetic manipulations and/or endogenous/exogenous toxic insults to recapitulate the symptomatology and/or neuropathology of PD [4]. Thus far, the standard of animal model behavioral assessment has been the presence of a Parkinsonian motor phenotype [5,6]. Various behavioral tests have been routinely used to qualify PD mouse models including, locomotor

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activity, rotarod, forepaw stride length, grid test, and pole test [5-10] (see Table 1). While these techniques have proven useful for verifying a Parkinsonian motor phenotype and gaining insight to possible causes of motor dysfunction, a disconnect still remains between the wealth of mouse models for PD and the slow progress towards restorative therapeutics. This disconnect may be due to the evolving definition of PD as a multisystem syndrome [11-13].

Clinically, PD is not diagnosed until the onset of motor deficits [11,14]; this has likely contributed to the dominance of motor-based animal models. However, there are many non-motor symptoms associated with PD that can appear years, sometimes decades, before the onset of the motor phenotype [15-17]. These symptoms include hyposmia, sleep abnormalities, gastrointestinal disturbances, anxiety, depression, autonomic dysfunction, and impaired cognition [15-17]. Some of these symptoms respond to dopaminergic therapies; others do not and contribute to diminished quality of life for PD patients [15,18,19]. This highlights the importance of shifting attention to non-motor symptomatology in mouse models of the disease. The non-motor phenotype is more difficult to treat as the underlying pathophysiology remains unclear. This may be a reflection of the widespread neuronal loss that occurs in neurotransmitter systems other than the nigrostriatal pathway [12,14]. Though, at present, the effects of nondopaminergic drugs in mouse models do not effectively translate into clinical efficacy [13,16,20]. Expanding the required behavioral phenotype in mouse models could lead to progress towards more effective therapeutics. Here, we propose a battery of behavioral tests designed to assess a larger array of behavioral symptoms.

General Health

Although Parkinson's disease is a debilitating disorder with symptoms spanning a wide spectrum of organ systems, there are many aspects that remain healthy in PD patients. When characterizing a mouse model, it is important to confirm that any aberrant phenotypes are not due to general poor health. For example, after acute administration of MPTP or rotenone animals become quite ill. It is only after the animals have had the chance to recover from the acute toxicity that PD-like behaviors can be evaluated. Moreover, PD is a relatively selective disorder in that it primarily targets regions innervated by monoamines [11,21]; even within this category some symptomatology remains rare. While characteristic phenotypic behaviors help define the disease, lack of deficits in other systems is also important in showing relative selectivity.

Tactile, gustatory function and trigeminal nerve response are used as indices of gross sensory function, independent of olfactory deficits. Responsiveness to tactile stimulation is commonly assessed by latency to remove a small adhesive dot from the animal's forehead [9,22,23]. In a two minute trial, an adhesive dot is placed between the ears on the top of the head in their home cage and monitored for latency to removal. Despite dopamine abnormalities, dopamine transporter knockout (DAT -/-), D_2 receptor knockout (D_2 -/-), and vesicular monoamine transporter 2 (VMAT2) deficient mice, all of which have some level of altered dopamine homeostasis, were found to respond normally to tactile stimulation relative to their wildtype littermates [22,23].

Gustatory function can be tested using a taste aversion paradigm. Quinine is frequently used due to its unpalatable bitterness [22,23]. In this test, a cotton swab is used to expose the mouse to either an aliquot of quinine or water; the latency to groom or drag the jaw along the ground is then measured during a one minute test session [22,24,25]. Again, despite abnormalities in dopamine and other monoamines, DAT -/-, D_2 -/-, and VMAT2-deficient mice displayed similar taste aversion to quinine as their wildtype littermates [22,23]. Finally trigeminal nerve function is assessed by exposing the mice to ammonia, which is a known irritant, or water. While the trigeminal nerve innervates the olfactory epithelium and nasal mucosa, it is not

responsible for olfactory responses. Rather it is responsible for non-odor sensations such as mild irritation and burning [22]. In a two minute session, the animal's response to ammonia or water is measured by time spent sniffing (nose <1 cm away from stimulus) each scent. Normally mice show preferential exploration of water due to the mild irritation induced by ammonia [22,23]. Collectively, these three simple tests can indicate deficits in general sensory function, independent of any perceived olfactory deficits.

Finally, even though vision has not been found to be significantly affect in PD, normal vision is a prerequisite for many behavioral tests, especially for visuospatial learning tasks. For more general tests of visual acuity, the visual cliff test can be employed. Visual cliff measures the ability of a mouse to see the drop-off at the edge of a horizontal surface [26]. The test consists of a box with a ledge covered with patterned contact paper to emphasize the drop-off; a piece of clear plexiglass covers the ledge so there is only a perceived drop-off. If a mouse is blind, it will not see the appearance of the edge and explore the plexiglass immediately [26]. Visual cliff serves to assess gross visual function and give insight as to if more complex visual tests should be completed. Gross visual function can be quantified by electroretinogram (ERG). The ERG can detect abnormalities in retinal function and in the electrical responses of photoreceptor cells [27,28]. Studies in younger VMAT2-deficient mice have demonstrated zero deficiencies in retinal function as compared to wildtype littermates [23].

Motor phenotype of PD

Parkinson's disease is not usually diagnosed until over 80% of the striatal dopamine innervation [15,20,29]. When the neurons in the SNpc die, the levels of dopamine in the striatum decrease resulting in a loss of voluntary motor control. The cardinal symptoms of PD include tremor, rigidity, postural instability, and bradykinesia, which have contributed to the basis of most behavioral testing in mouse models of PD. The most prominent model of PD was discovered because of its ability to recapitulate many of the features associated with the severe motor phenotype of PD [30]. One of the most basic ways to assess the presence of a motor phenotype in a mouse model of PD is to use locomotor activity chambers. Mice are typically placed in transparent locomotor chambers, and activity is measured by consecutive photobeam breaks or ambulations in a certain time period. As shown in Table 1, most mouse models of PD reflect a basic motor phenotype as evidenced by decreased locomotor activity in open field behavioral chambers. When challenged with L-DOPA, the LRRK2, Pitx3-aphakia, MitoPark, and VMAT2-deficient mice all displayed increases in locomotor activity [31-34]. The shuffling gait observed in PD patients can be considered analogous to forepaw stride length, which is also easily evaluated in mouse models of PD. In this test, mice are trained to walk down a narrow corridor and their forepaws are inked to analyze any deficits in stride length [7]. The α-synuclein transgenic mice, DJ-1 mice, and mice dosed with MPTP or reserpine all demonstrate deficits in forepaw stride length; however, only MPTP-dosed and reserpinized mice respond positively to acute doses of L-DOPA [7,35-38]. Other features of the Parkinsonian motor phenotype such as coordination, rigidity, and tremor can also be tested behaviorally and are outlined in Table 1.

Modeling PD in animals has, until recently, focused on behaviors that involve striatal function and that should improve with dopamine replacement therapy [39]; and previously, criteria for a functional PD animal model only demanded a motor phenotype. While all Parkinsonian behaviors should be tested for their response to L-DOPA, it is paramount that the primary motor deficits observed in mouse models respond positively to this primary PD therapeutic. However, research suggests that PD is more than a dopamine-based motor disorder, thus exploring non-motor symptoms and non-dopamine mediated behaviors is warranted. By using a more comprehensive battery of behavioral tests, new pharmacological treatments can be developed that treat more than the dopaminergic deficit (see Table 3).

Olfactory deficits in PD

Since that the preclinical phase of PD begins long before the degeneration of the substantia nigra, assessment of non-motor symptoms can further the understanding of the true PD disease process. Olfactory disturbances are one for the first non-motor symptoms observed in PD; hyposmia can be multifaceted and are not restricted to one modality. PD patients have demonstrated impairments in odor detection, differentiation, and identification [40-42]. Moreover, this non-motor symptom is not responsive to dopaminergic therapies [43], and occurs with a similar frequency to resting tremor [40]. Behavioral testing of olfaction can facilitate an earlier detection of PD, since impaired olfaction has been positively correlated with an increased risk of developing the disease [44].

To measure general olfactory function, the buried pellet test can be used. This test relies on the mouse locating a hidden object, usually a food pellet, by odor [26,45,46]. The amount of time a food-restricted animal takes to find and uncover a food pellet or reward is measured. Alternately, the latency to locate buried food versus the latency to locate food placed on the surface can also be measured [45]. In a study with mice overexpressing human wildtype α-synuclein (Thy1-aSyn), Thy1-aSyn mice displayed a longer latency to find a buried pellet than wildtype littermates [45] (see Table 2). However, Thy1-aSyn mice have a similar latency to wildtype mice when forced to locate a food pellet on the surface [45]. Studies using MPTP-treated mice and ApoE knockout mice yielded similar results [46,47].

The novel scent test and block test can be used to measure olfactory acuity and discrimination. The novel scent test is a simple way to quantify time spent sniffing/exploring a novel odor. This test can be made more relevant to human PD by using scents tested in the University of Pennsylvania Smell Identification Test, which is commonly used to diagnose hyposmia/anosmia in humans [48]. Commonly used olfactory cues are attractive to mice, but vary over a range of scent classes. The mouse is presented small aliquots of either a novel scent (lemon, peppermint, or vanilla) or water simultaneously [23]. Time spent sniffing each odor is recorded for a three minute session. When given the choice between a novel odor and water, both VMAT2 wildtype and deficient mice show a preferential exploration of the novel scent at 2 months of age; however, VMAT2-deficient mice lose this ability by 18 months of age [23] (see Table 2). The block test evaluates the ability of mice to discrimination between social odors, specifically self and non-self [22,23]. The animal is presented with a wooden block scented with its own bedding and a block scented with another mouse's bedding (of the same sex). The time spent in contact with each block is recorded for a two minute trial [22,23]. In experiments with DAT knockout and D_2 knockout animals, DAT and D_2 -/- mice do not display a preferential exploration of the block scented with a foreign animal's bedding, which was not due to decreased exploratory activity [22]. This is in contrast to wildtype littermates that always display a preferential exploration for the block scented with a foreign animal's bedding [22]. Similarly, VMAT2 wildtype mice show preferential exploration of the block scented with the foreign animal's bedding at all ages; whereas, VMAT2-deficient mice exhibit preferential exploration until 4 months of age, but not by 6 and 12 months of age [23] (see Table 2).

To examine more subtle olfactory deficits, habituation/dishabituation paradigms can be employed. Briefly, a small plastic cartridge is packed with cotton scented with a novel odor and placed in the home cage of the subject for one minute over four trials. In the fifth dishabituation trial the subject is presented with a cotton ball scented with a different novel odor. The time spent in olfactory investigation for each trial is recorded, and olfactory investigation is defined as direct nasal contact with the cartridge [45,49]. DAT and D_2 -/- mice were both able to habituate to the novel odors of paprika or cinnamon, but neither mouse demonstrated increased investigation to novel odor versus the habituated odor, unlike their

wildtype littermates [22]. Thy1-aSyn mice were able to habituate and then discriminate between habituated and novel odors at younger ages [45] (see Table 2).

Sleep abnormalities in PD

Nocturnal sleep disturbances affect 60-98% of patients suffering from Parkinson's disease, including night-time awakenings, sleep fragmentation, and REM sleep disorder [50]. Because PD patients often do not get a full night's sleep, they also suffer from excessive daytime sleepiness (EDS). EDS has also been found to increase the risk for development of PD and is correlated with advanced stages and longer duration of PD [51,52]. Sleep dysfunction is an important non-motor symptom associated with PD and has been correlated with other non-motor symptoms such as anxiety, depression, and GI disturbances [53,54]. These problems occur more frequently as the PD disease state advances; early untreated patients often report nocturia, night-time cramps, dystonia, and tremor [15,54,55]. However, some night-time problems can be reduced by optimizing PD medications, since motor symptoms can contribute to disturbed sleep and EDS [55].

Measuring latency to behavioral signs of sleep is the simplest test used to evaluate sleep abnormalities in mice. This test assesses the time it takes for mice to attain behavioral signs of sleep during their circadian nadir. During sleep, mice exhibit a distinctive posture and breathing pattern that allows the observer to determine onset [56]. On test day, mice are removed from their home cages, placed individually in behavioral chambers, and allowed to acclimate for 4 hours during their light cycle. During circadian nadir, mice are handled, injected with saline to ensure they are awake, placed back into the behavioral chamber and monitored for latency to achieve behavioral signs of sleep [23,56]. Sleep is defined as two minutes of uninterrupted sleep behavior, and 75% of the next 10 minutes spent asleep; this behavioral scoring paradigm has been shown to reliably correlate with onset of sleep using electroencephalography (EEG) measurements [56,57]. Although this test has not been completed in many mouse models of PD, sleep latency has been conducted in a mouse model of catecholamine deficiency. Dopamine β-hydroxylase knockout (*Dbh* −/−) mice, that lack norepinephrine after birth, have a shorter sleep latency than *Dbh* +/− mice, which have wildtype noradrenergic levels [56,58]. Treating Dbh -/- mice with the wakefulness promoting drug, modafinil, dose-dependently increases sleep latency in both *Dbh* +/− and *Dbh* −/− mice, but the *Dbh* −/− mice were hypersensitive to the wake-promoting effects of modafinil [56].

If a mouse model displays an altered latency to behavioral signs of sleep, more sophisticated tests involving polysomnography and electromyography (EMG) can be employed. Polysomnography can help determine the underlying characteristics of sleep abnormalities, and which stages of the sleep-wake cycle may be affected. These studies are most similar to methods used to detect aberrant sleep phenotypes in humans [59,60]. EMG electrodes are inserted into the neck muscles and the mouse is recorded for 24-48 hour periods. Polysomnographic recordings are then scored visually in five second epochs as wakefulness, slow wave sleep (SWS), or paradoxical sleep (PS) according to standard criteria [61,62]. Studies in mice that were exposed to MPTP 20 days prior reveal increased PS during the dark phase and increased number of PS bouts during a 24 hour period compared to mice dosed with vehicle [60,63]. When MPTP mice were treated with an acute dose of L-DOPA, PS latency and amounts of wakefulness were found to increase [63]. However, 40 days after MPTP exposure, no differences between treated and control mice were observed in the number of PS episodes during the dark cycle or in a 24 hour period [60] (see Table 2).

An alternate test to polysomnography is using a 2-dimensional state map with EMG to identify behavioral sleep states. This test is used to differentiate rapid eye movement (REM) sleep specifically from wakefulness, and is confirmed using behavioral observations [64]. Novelty

exposed DAT -/- mice were observed to enter a novel awake state resembling REM sleep [64]. Interestingly, when DAT -/- mice are acutely depleted of dopamine, they enter a different novel awake state, which resembles SWS, but with suppression of SWS and REM sleep [64]. Treatment with D_2, but not D_1, agonists recovers only REM sleep [64]. Despite these sophisticated sleep behavioral paradigms, the underlying causes of sleep disorders in PD remain unknown and could be due to disease progression, dopaminergic or noradrenergic pathology, current medications, or a combination of factors.

Gastrointestinal dysfunction in PD

Gastrointestinal (GI) dysfunction affects more than 70% of PD patients and has been attributed to a variety of factors including lack of activity, inadequate hydration, and autonomic/enteric neuronal dysfunction [65-67]. Pathologically, Lewy bodies have been found in the myenteric and submucosal plexuses of the enteric nervous system [68-70]. Moreover, there is evidence of decreased dopamine neurons in the enteric nervous system of PD patients, and for the involvement of the dorsal motor nucleus of the vagus (DMV) due to α-synuclein pathology independent of nigral degeneration [21,71,72]. Some manifestations of GI dysfunction in PD are early satiety and nausea from delayed gastric emptying, bloating from poor small bowel coordination, and constipation [68]. However, GI dysfunction is not exclusive to late stage PD patients, and can also manifest in the early stages of the disease before motor involvement [73]. Delayed gastric emptying can also interfere with drug action by disrupting drug absorption in the intestinal tract [67]. An association between the frequency of bowel movements and risk for developing PD has been found in patients who are constipated, defined as having fewer than three bowel movements in a week; these patients have 2-7 times higher risk of developing PD later in life [66,67].

Screening for GI dysfunction in mouse models of PD can be done using solid gastric emptying to evaluate stomach motility and stool collection to examine colon motility. Solid gastric emptying is tested after the mouse undergoes a 12-hour fast. Mice are then allowed free access to food for a defined period and, the amount of food consumed is calculated. After food removal (15-120 minutes), animals are killed, the stomach contents are weighed (wet and dried), and the percentage of food remaining in the stomach is measured [23,68,74]. When compared to wildtype animals, VMAT2 deficient mice were found to have delayed gastric emptying overall, with a greater apparent effect at 12 months of age [23] (see Table 2). MPTP has been shown to deplete dopamine neurons in the enteric nervous system; however, solid gastric emptying remained unaffected [68] (see Table 2).

To screen for constipation in mouse models, the mouse is monitored for one hour and stool is collected. Each mouse is placed in a separate clean cage and observed throughout the 60 minute collection period. Fecal pellets are collected immediately after expulsion and placed in sealed (to avoid evaporation) 1.5 mL tubes. Tubes are weighed to obtain the wet weight of the stool; these are then dried overnight at 65°C and reweighed to obtain the dry weight [23,68,75]. Even though PD patients have been found to be constipated, several mouse models of PD have demonstrated increased stool frequency compared to wildtype or saline treated animals. Thy1-αSyn mice exposed to a novel environment had an increased stool frequency relative to wildtype mice; although, when habituated to the experimental environment Thy1-aSyn mice displayed reduced stool frequency [76] (see Table 2). Correspondingly, mice treated with MPTP have significantly higher stool frequency 2-3 days after treatment, which decreases to saline-treated animals' levels by 8-10 days after MPTP treatment [68] (see Table 2). Similar results were observed in VMAT2-deficient mice [23] (see Table 2).

Colon motility and innervation can be monitored in more detail using bead latency and isometric muscular force recording [68,77]. In the bead latency test, mice are anesthetized and

a glass bead is inserted into the colon; distal colon motility is assessed by monitoring the time required for the bead to be expelled [76]. To evaluate enteric neuronal circuitry, sections of proximal colon are removed with their enteric innervation intact and suspended between electrodes in Krebs buffer. Myenteric neuron function can be interrogated using a combination of pharmacological agents and electrical field stimulation using muscular force as a readout [68,77]. Bead latency was found to be unchanged in Thy1-αSyn mice, compared to wildtype littermates [76] (see Table 2). In mice treated with MPTP, enteric dopamine neuron loss was found to impair relaxation of muscle from the proximal colon, indicating dysfunction in the inhibitory neurons in the enteric nervous system of MPTP-treated animals [68] (see Table 2).

Other assays of total GI transit time and small intestine transit can also be considered. As with most non-motor symptoms, since the exact cause of GI dysfunction in PD patients remains unknown, interpretation of gastrointestinal dysfunction and response to therapeutic interventions in mouse models should be cautious.

Anxiety and Depression

Anxiety and depression affect approximately 40% of patients with PD; however, the reason for such a high frequency of these two disorders in PD is poorly understood [78-80]. The rate of severe depression in PD has been found to be twice that of other equivalently disabled patients, but because of overlapping clinical symptoms, diagnosis of anxiety and depression is subjective [78,80]. Moreover, even though there is a significant association between phobic anxiety scores, depressive episodes and risk of developing PD, neither disorder parallels PD pathogenesis or progression [78,81,82]. Although depressed PD patients have corresponding loss in monoaminergic (dopaminergic, noradrenergic, and serotonergic) projections, mood fluctuations have been found to occur independently of motor fluctuations and are often improved by anti-PD medications [79,83,84]. Clinical features of depression in PD include increased levels of dysphoria, irritability, feelings of failure, but low suicide rates and ideations of suicide [79,80,85]. Even though anxiety and depression are under-diagnosed and undertreated in PD, they have a major impact on quality of life in PD [79,80,86].

Anxiety-like behaviors can be measured effectively in mice by using the elevated plus maze (EPM), open field testing, and light-dark exploration. The EPM apparatus consists of two open arms and two enclosed arms arranged in a plus-sign orientation. Because rodents naturally prefer dark, enclosed compartments, a greater willingness to explore the open, well-lit arms is believed to represent a decrease in the animal's anxiety [87-89]. DJ-1 knockout mice were found not to exhibit an anxiety-like phenotype as measured by EPM, showing no significant difference in exploration times compared to wildtype littermates [38] (see Table 2). Alpha-synuclein (A53T) transgenic mice displayed reduced anxiety-like behaviors on the EPM at younger ages [90] (see Table 2). Most notable, the α-synuclein transgenic mice demonstrated significantly higher time spent in the open arms of the EPM compared to wildtype and α-synuclein knockout mice [90]. Interestingly, younger VMAT2-deficient animals spend a greater percentage of their time in the closed arms of the elevated plus maze as compared to age-matched WT animals, exhibiting an anxiety-like phenotype [23] (see Table 2).

In the open field test, mice are placed into an open behavioral chamber, usually during the light cycle, where horizontal and vertical activities can be monitored by photobeam breaks [91]. Moreover, the animal's behavior can be videotaped and analyzed for time spent in the center of the chamber versus along the perimeter [92]. Since rodents have a natural inclination to stay near the perimeter of a chamber (thigmotaxis), increased thigmotaxis is indicative of anxiety-like behavior [92]. Although, the A53T α-synuclein transgenic mice did not have an anxiety-like phenotype in the EPM, the α-synuclein transgenics did display a selective anxiety-like phenotype in the open field test [90] (see Table 2). This was indicated by reduced habituation

and increased thigmotaxis compared to α-synuclein knockout and wildtype mice [90]. Parkin-deficient mice also exhibit increased thigmotaxic behavior, coupled with decreased horizontal travel distance, compared to wildtype mice [93] (see Table 2).

The light-dark exploration test is frequently used to further analyze anxiety-like phenotypes in mice. The test is conducted in a behavioral chamber partitioned into two compartments. One compartment is clear without a lid and illuminated by an overhead lamp, while the other compartment is enclosed with black cloth and covered with a black plexiglass lid. Mice are placed in the illuminated side, and, after a period of time, are allowed access to the darkened side of the chamber. Latency to first enter the darkened side, time spent on each side, head pokes into each side, and number of transitions between chambers can all be used to assess anxiety-like behavior [91,93]. Both younger and older parkin mice were found to spend significantly less time in the illuminated side of the light-dark chamber and made fewer transitions between the two compartments than wildtype littermates [93] (see Table 2). Taken together, these results are indicative of an anxiety-like phenotype that does not appear to be age-dependent [93]. However, mice treated with MPTP were not found to exhibit an anxiety-like phenotype as measured by the light-dark exploration test [94] (see Table 2).

Depression is one of the most difficult behaviors to assess in mouse models, and most tests are actually tests of antidepressant efficacy. The forced swim test and tail suspension tests are acute measures of antidepressant efficacy that rely on immobility or behavioral despair to determine depressive-like behavior in mice. This immobility may be related to variations in stress-induced behavioral depression and can be correlated with the psychological construct of entrapment seen in clinical depression [95,96]. In the forced swim test, mice are placed individually in glass cylinders with 6 inches of water, and their behavior is videotaped from the side of the cylinder for 6 minutes. After the first two min, the total duration of time spent immobile is recorded during a 4 minute test period [91,97]. VMAT2 heterozygous mice, which have a 50% decrease in VMAT2 expression, have significantly increased immobility times compared to wildtype animals, suggesting a depressive-like phenotype [91]. When given the antidepressant imipramine, immobility times were reduced to those of wildtype mice [91]. Similarly, VMAT2-deficient mice, which have a 95% decrease in VMAT2 expression, display an age-dependent depressive-like phenotype demonstrated by an increased immobility time, which is decreased to WT levels when dosed with desipramine [23]. In the tail suspension test, mice are individually suspended by the tail from a horizontal ring stand bar (distance from the floor = 30 cm) using adhesive tape. A six minute test session is then videotaped and scored by a trained observer for escape-oriented behavior and bouts of immobility; mice are excluded from the test if they climb up their tail during the test session [98]. As in the forced swim test, VMAT2 heterozygous mice exhibit increased immobility times compared to wildtype littermates, which are reduced to wildtype levels when dosed with fluoxetine, reboxetine or bupropion [91]. VMAT2-deficient mice have an age-dependent increase in immobility time in the tail suspension test compared to wildtype littermates, which is ameliorated with desipramine [23]. However, mice exposed to MPTP did not demonstrate a significant difference in immobility time from saline-treated animals in the tail suspension test [94] (see Table 2).

To study anhedonia associated with depression in PD, the sucrose preference test can be employed. This test is conducted in the animal's home cage and the mouse is habituated to consume a palatable, weak sucrose solution. Prior to the test, mice are deprived of water overnight [91,94]. During the test period, two bottles, one containing water and one containing the sucrose solution, are presented to the animal. Consumption is measured by weighing the bottles before and after the test period, and preference for the sucrose solution is determined by dividing the volume of sucrose consumed by the total liquid (water and sucrose) consumed [91,99]. The concentration of sucrose can also be varied over consecutive test days to assess the degree of sucrose preference, and therefore, the degree of anhedonic phenotype [91].

Reserpinized mice display a significant decrease in sucrose preference compared to vehicle treated mice [99] (see Table 2). Since reserpine is known to elicit locomotor deficits, total liquid consumption was also measured; no differences were observed in total liquid consumption between vehicle and reserpine-treated animals [99]. VMAT2-heterozygous mice were also found to have reduced preferences for various concentrations of sucrose solution compared to wildtype littermates, suggesting an anhedonic phenotype when VMAT2 expression is decreased [91]. Alternatively, MPTP treated mice do not display any differences in sucrose preference compared to vehicle treated mice [94] (see Table 2).

Cognitive deficits in PD

PD patients experience a myriad of cognitive deficits, including, but not limited to, impairments in executive functions, language, memory, visuospatial skills, and dementia [100-102]. To test for these deficits in mouse models of PD, primarily memory and general cognitive tests are used as it is hard to measure executive function in mice. The radial arm maze functions to assess the mouse's ability to remember a set of spatial locations based on memory and not response patterns. The maze consists of eight arms and a central platform, with food rewards located randomly among the 8 arms [103]. To increase motivation in learning the maze correctly, a 15% reduction in daily food intake is enforced. During the test sessions, four randomly selected arms are baited with one pellet of food each; the baited arms are kept unchanged throughout the experiment [103,104]. After each test session, the mice are evaluated for three parameters: working memory errors, reference memory errors, and total arm entries. Working memory errors are classified as reentries into baited arms that had been previously entered during the test session, and reference memory errors are classified as entry into non-baited arms [104,105]. The mice were considered to have learned the task when the number of working memory errors reached zero and the number of reference memory errors is one [103,105].

The modified Morris water maze is an analogous test using visual cues to measure spatial learning and memory in mouse models. Spatial learning is evaluated using a circular water tank filled with water made opaque by the addition of milk or non-toxic paint to obscure an escape platform [93,106,107]. The tank is divided into 4 quadrants, with designated start positions in each quadrant. During training sessions, the escape platform is made visible by the attachment of a flag; the mice were subsequently trained to swim to the submerged platform without the flag [93,106]. Mice are allowed one minute to locate the escape platform, and escape latencies are recorded. On test day, the escape platform is removed, and the swimming path is recorded while the mouse searches for the missing platform [93]. Parkin-deficient mice have been found to exhibit mild cognitive impairment, indicated by longer escape latencies and failure to selectively cross into the quadrant that formerly contained the escape platform [93] (see Table 2).

Conclusions

In addition to motor deficits, there are a variety of non-motor symptoms associated with PD. These symptoms may precede the onset of motor symptoms, sometimes by years, and include anosmia, problems with gastrointestinal motility, sleep disturbances, cognitive deficits, anxiety, and depression. As research in the field advances, non-motor symptoms associated with Parkinsonism have been illuminated, demonstrating that PD is not exclusive to dopaminergic disturbances; norepinephrine (NE), serotonin (5-HT), and non-aminergic transmitter systems may significantly contribute to disease progression as well. To complement the expanding definition of Parkinson's disease, new animal models of the disease should encompass more than motor deficits. Most studies of widely-accepted mouse models of PD only explore motor deficits; very few begin to assess non-motor behaviors that are highly

prevalent within the disease (see Table 2). Mouse models that display overt pathology but lack behavioral deficits must be interpreted with caution. It is very important to acknowledge the possible relationships between neurodegeneration, behavioral outcome, and potential therapeutic interventions. By testing potential mouse models of PD for a broader behavioral phenotype, conceivably the etiology of PD can be better understood, leading to earlier diagnosis and improved therapeutic strategies.

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Table 1

L-DOPA responsive motor phenotypes in Parkinsonian mouse model

Mouse Model	General Activity (open field, novelty-induced locomotor activity)	Coordination (pole test, challenging beam traversal, rotarod)	Gait (stride length, grid test, treadmill)	Other (AIMS)
MPTP	↓locomotion [108]	↓pole test [109], ↓rotarod performance [110] +L-DOPA	↓stride length, performance on grid test [7] +L-DOPA	↑ catalepsy, akinesia, tremor [111-113]
Rotenone	↓locomotion [114,115]	↔rotarod [115]	↓stride length [115]	↑ catalepsy [115]
Paraquat	↓locomotion [114,116]	↓ pole test [116]	N.P.	N.P.
Paraquat + Maneb	↓locomotion [117]	↓ challenging beam performance, inverted screen performance [117]	N.P.	N.P.
Reserpine	↓locomotion [118]	N.P.	↓stride length [35] +L-DOPA	↑ akinesia [35]
A53T Synuclein Transgenics	↑ locomotion [119]	↔rotarod [120]	↓stride length [36]	N.P.
A30P Synuclein Transgenics	↓locomotion[121,122] -L-DOPA	↓challenging beam performance [37], ↔rotarod [122]	↓stride length [37]	↑dystonia, rigidity [123] -L-DOPA
Thy1-aSyn	N.P.	↓challenging beam performance, pole test [124] -L-DOPA	↔ stride length [124], ↔ grid test [125] -L-DOPA	↔ rearing, grooming [124] -L-DOPA
Parkin	↔locomotion [93,126]	↔rotarod [93,126], ↔ pole test, challenging beam [126,127]	↔ stride length, grid test [127]	↔ catalepsy test [127]
LRRK2	↓locomotion [31] +L-DOPA	N.P.	N.P.	↓rearing [31] +L-DOPA
UCH-L1	N.P.	N.P.	N.P.	N.P.
PINK1	↓locomotion [128]	↔rotarod [128,129]	N.P.	N.P.
DJ-1	↓locomotion [38,130]	↔pole test [38], ↔rotarod [130]	↓stride length [38], ↑foot faults in grid test [131] -L-DOPA	N.P.
Pitx3-aphakia	↔locomotion, ↓exploratory activity [32] +L-DOPA	↔rotarod [132], ↓challenging beam traversal and pole test performance [32] +L-DOPA	N.P.	N.P.
MitoPark	↓locomotion, exploratory behavior [33] +L-DOPA	N.P.	N.P.	↑ tremor, rigidity [33]
VMAT2-deficient	↓locomotion [34] +L-DOPA	↓challenging beam performance [133]	↓stride length* +L-DOPA	N.P.

+/- L-DOPA: L-DOPA efficacy or inefficacy in rescuing motor phenotype, respectively, N.P.: test not performed

*Taylor and Miller, unpublished observations

Table 2

Behavioral phenotypes in mouse models of Parkinson's disease

Mouse Model	Olfactory Disturbances	Sleep Abnormalities	GI Dysfunction	Anxiety	Depression	Cognitive Decline
MPTP	+, buried pellet [47]	+, polysomnography [60, 63]	-, gastric emptying, + stool frequency [68] + colonic motility [68]	-, light-dark exploration [94]	-, TST, sucrose preference [94]	+, T-maze [134]
Rotenone	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.
Paraquat	N.P.	N.P.	N.P.	+, open field [135]	N.P.	N.P.
Paraquat + Maneb	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.
Reserpine	N.P.	N.P.	N.P.	N.P.	+ sucrose preference [99]	+, discriminative avoidance task [136]
A53T Synuclein Transgenics	N.P.	N.P.	N.P.	-, EPM; + thigmotaxis, open field [90]	N.P.	N.P.
A30P Synuclein Transgenics	N.P.	N.P.	N.P.	N.P.	N.P.	+, Morris water maze, fear conditioning [137]
Thy1-aSyn	+, block test, habituation/ dishabituation [45]	N.P.	+ colonic transport, stool frequency [76]	N.P.	N.P.	N.P.
Parkin	N.P.	N.P.	N.P.	+, light-dark exploration, - open field [93] -, EPM [127]	-, FST, TST [127]	+, Morris water maze [93], T-maze [138]; -, novel-object recognition [127]
LRRK2	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.
UCH-L1	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.
PINK1	N.P.	N.P.	N.P.	-, open field [128]	N.P.	N.P.
DJ-1	N.P.	N.P.	N.P.	-, EPM [38]	N.P.	N.P.
Pitx3-aphakia	N.P.	N.P.	N.P.	N.P.	N.P.	+, T-maze [132]
MitoPark	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.
VMAT2-deficient	+, novel scent test, block test [23]	+, sleep latency [23]	+stool frequency, gastric emptying [23]	+, EPM [23]	+, FST, TST [23]	N.P.

+, evidence of symptom; -, no evidence of symptom, N.P., not performed

Table 3

Summary of Parkinsonian behavioral analyses

PD symptom	Preliminary Behavioral Assays	Comprehensive Behavioral Assays
Hyposmia/Anosmia	Buried pellet test, novel scent test, social olfactory discrimination (block test)	habituation/dishabituation
Sleep Abnormalities	sleep latency	polysomnography, EEG
GI Dysfunction	stool frequency, solid/liquid gastric emptying	colonic motility, bead latency, isometric muscular force recording
Anxiety	elevated plus maze, open field, light-dark exploration	novelty suppressed feeding
Depression	forced swim test, tail suspension test, sucrose preference	learned helplessness
Cognitive Decline	novel object recognition, radial arm maze, T-maze	Morris water maze, fear conditioning, discriminative avoidance task
Motor abnormalities	open field, novelty-induced locomotor activity, stride length	challenging beam traversal, pole test, grid test, catalepsy test