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A chronic mouse model of Parkinson's disease has a reduced gait pattern certainty

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Abstract

The purpose of this investigation was to determine if a chronic Parkinson's disease mouse model will display less certainty in its gait pattern due to basal ganglia dysfunction. A chronic Parkinson's disease mouse model was induced by injecting male C57/BL mice with 10 doses of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (25 mg/kg) (MPTP) and probenecid (250 mg/kg) (P) over 5 weeks. This chronic model produces a severe and persistent loss of nigrostriatal neurons resulting in dopamine depletion and locomotor impairment. The control mice were treated with probenecid alone. Fifteen weeks after the last MPTP/P treatment, the mice were videotaped in the sagittal plane with a digital camera (60 Hz) as they ran on a motorized treadmill at a speed of 10 m/min. The indices of gait and gait variability were calculated. Stride length was significantly ($p = 0.016$) more variable in the chronic MPTP/P mice. Additionally, the chronic MPTP/P mice had a statistically less certain gait pattern when compared to the control mice ($p = 0.02$). These results suggest that variability in the gait pattern can be used to evaluate changes in neural function. Additionally, our results imply that disorder of the basal ganglia results in less certainty in modulating the descending motor command that controls the gait pattern.

Keywords: Variability; Gait; Locomotion; Parkinson's disease; Entropy; MPTP; Probenecid

Parkinson's disease is a neurological disorder involving the basal ganglia that progressively diminishes the function of the motor system [11]. It has been well documented that as the disease progresses there is a reduction in gait speed, shortening of the step length, and a narrowing of the base of support [11]. Additionally, recent gait studies have indicated that individuals suffering from Parkinson's disease have an increased amount of variability in the stepping pattern [3,7,15]. Variation in the stepping pattern of human movement appears to be related to the certainty of the nervous system in selecting a motor command for a stable gait [8]. A lack of certainty may arise from the inability of the nervous system to properly integrate the sensory and motor mappings that govern the descending motor command [16]. The basal ganglia's role in the motor integration process involves modulating the amplitude and timings of motor command that controls the stepping pattern [7]. Potentially, the increased gait kinematic variability seen in individuals with Parkinson's disease may be related to the uncertainty of the basal ganglia to properly modulate the descending motor command. Such uncertainty may progressively worsen with the manifestation of the disease, and may be related to an increased incidence of falls found in the Parkinson's disease population [15].

Animal models of Parkinson's disease have been frequently used to provide further understanding of the disease pathophysiology and for the exploration of new therapies [14]. A common method for creating a mouse model of Parkinson's disease is to repeatedly administer 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [4]. MPTP selectively destroys the nigrostriatal dopaminergic neurons and results in acute or subacute affects that resemble some of the features of Parkinson's

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Table 1
Features of Parkinsonism demonstrated in the chronic MPTP/P mouse model

Measurement	Characteristics
Striatal dopamine content	>90% loss for at least 6 months [13]
Striatal terminal dopamine uptake	>90% inhibited for at least 6 months [13]
Substantia nigra pars compacta cells	Early neuronal apoptosis and >50% loss of tyrosine hydroxylase positive cells [12,13]
Animal locomotor behavior	Deficit in rotarod performance and amphetamine-stimulated behavior [1,13]
Pathological inclusions	Detection of murine form of inclusion bodies containing lipids, lysosomes, and are α-synuclein and ubiquitin-positive [10]

Mice (C57/BL) were treated with MPTP (25 mg/kg, s.c.) and probenecid (250 mg/kg, i.p.) twice a week for 5 weeks.

disease. A limitation of the acute and subacute MPTP mouse model is that its effects are spontaneously reversed over time, and the model is not closely correlated with the motor deficits or neuropathology seen in Parkinson's disease patients [13]. Lau et al. have developed a chronic mouse model of Parkinson's disease that uses a combination of MPTP and probenecid (P) [9,13]. Probenecid inhibits the urinary and neuronal clearance of MPTP which causes a dramatic loss of neurons in the substantia nigra and persistently impairs the dopamine transmission [9]. The chronic MPTP/P mouse model appears to have many features that are associated with Parkinson's syndrome, which are summarized in Table 1. In this investigation, we sought to further examine the hypothesis that the chronic MPTP/P mouse model has similar changes in gait variability as those noted in humans suffering from Parkinson's disease.

All animal protocols used in this investigation were approved by the Institutional Animal Care and Use Committee at the University of Houston, and were carried out strictly according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996). The mice were kept on a 12 h light/dark schedule, and had *ad libitum* access to food and water. Seven male, C57/BL mice (25–27 g) received 10 doses of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (25 mg/kg, s.c.) (MPTP) and probenecid (250 mg/kg, i.p.) (P) over 5 weeks as previously described [13]. Seven age-matched C57/BL control mice were treated with probenecid (250 mg/kg, i.p.) alone. Fifteen weeks after the last MPTP/P treatment, mice were videotaped in the sagittal plane with a digital camera (60 Hz) as they ran on a motorized treadmill at a speed of 10 m/min, 0° inclination. All gait data were collected on the same day and treadmill. The positions of the base of the tail, right fore and hind feet were manually digitized from the videos with commercially available motion capture software (*ViconPeak*, Centennial, CO). To account for any variation in the mouse's position on the treadmill we calculated the displacement of the feet relative to the base of the tail. These newly defined feet positions were used to determine the selected step and stride lengths. For this investigation, we evaluated 129 steps because this was the longest continuous gait trail that we could obtain from all mice. From the measured gait variables we calculated the coefficient of variation of the stride length [2]. This provided us with a general gait variability index that was similar to previous investigations of humans with Parkinson's disease [3,7,15]. The stride frequency (SF) was calculated from the step time series.

Statistical entropy was used to measure the nervous system's certainty in the selecting a step length [8]. It was assumed that the nervous system could select a number of different motor commands that would alter the selected step length. These step lengths were represented as discrete states of the locomotive pattern. Each of these states was associated with a probability (P_m) such that the sum of the probabilities for all the discrete states was equal to one. Eq. (1) expresses the probability of each discrete state examined:

$$P_m = \frac{|m|}{|S|} \tag{1}$$

where P_m was the discrete probability of a selected step length, $|m|$ the discrete state cardinality (*e.g.*, number of times the step length was repeated), and $|S|$ was the cardinality of the total discrete states observed. Based on the calculated probabilities, Eq. (2) was used to calculate the statistical entropy ($H(x)$) of the stepping pattern:

$$H(x) = -\sum_{m=1}^{m=129} P_m \log_2(P_m) \tag{2}$$

A small entropy value indicates a less amount of variability and greater certainty in the gait pattern. Conversely, a large entropy value indicates less certainty in the gait pattern [8].

Independent two-tailed *t*-test were performed to discern differences in the gait indices and gait variability measures of the control and MPTP/P group's gait patterns. Differences were considered significant with $P < 0.05$. Fig. 1 displays the gait pattern for a representative control (Fig. 1A) and MPTP/P mouse (Fig. 1B). General observations of the gait patterns suggest that the stepping pattern exhibited by the MPTP/P mouse was less consistent than the representative control mouse. Although it was not significant ($t[1, 12] = 1.88$; $p = 0.08$), the MPTP/P mice (SF = 1.8 ± 0.1 Hz) had a faster stride frequency than the control mice (SF = 1.6 ± 0.1 Hz). The MPTP/P mice (CV = 7.9 ± 2.1) pattern was statistically ($t[1, 2] = 2.89$; $p = 0.016$) more variable than the control mice (CV = 5.1 ± 1.6) The statistical entropy analysis indicated that the chronic MPTP/P mice ($H(x) = 5.11 \pm 0.2$ bits) had a statistically less certain gait pattern when compared to the control mice ($H(x) = 4.78 \pm 0.25$ bits) ($t[1, 12] = -2.56$; $p = 0.02$). Our results are similar to the gait deficits that have been noted in humans suffering from Parkinson's disease, and those found previously in an MPTP mouse model [2,3,7,15].

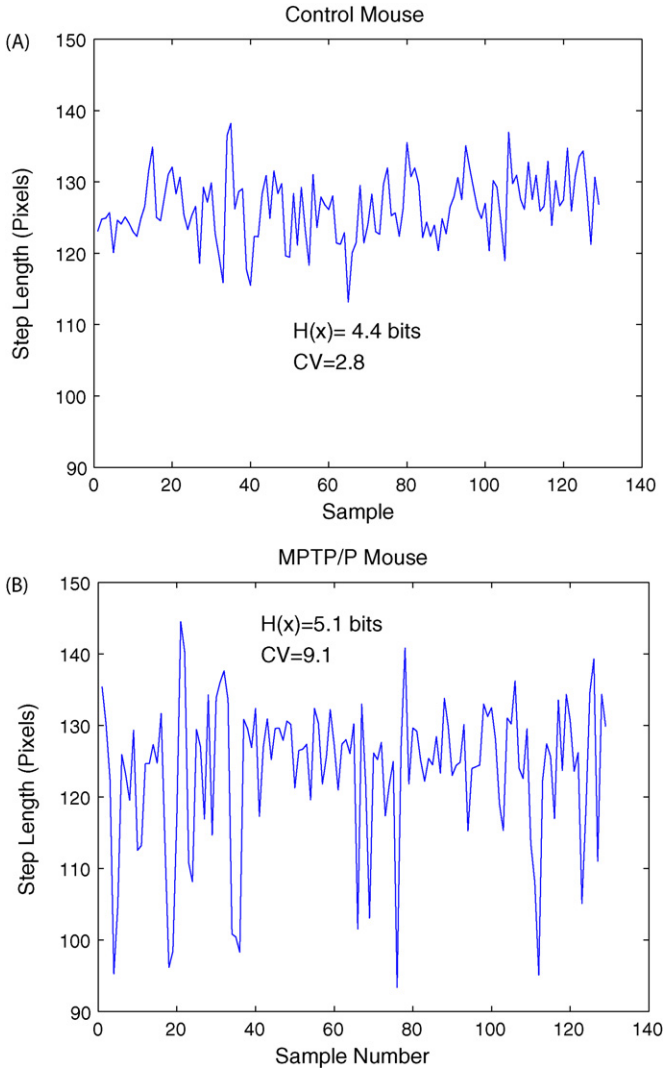

Fig. 1. Representative pattern of step length time series from a control mouse (A) and a chronic MPTP/P-treated mouse (B). Step length values are presented in pixels.

Generally, motor deficits in mouse models of Parkinson's disease have been studied by using measures of behavioral tasks, such as rotarod performance, balance beam or pole test [6]. Although these tests have documented motor impairments, it is questionable if these tests directly translate to the comparable declined motor performances noted in humans with Parkinson's disease since these tests are not clinically applied. The work presented here provides a new clinically relevant scientific approach for exploring the outcomes of a Parkinson's disease mouse model. Similar gait analysis techniques have recently been used to demonstrate that an MPTP mouse model has an increased amount of variability of the stepping pattern [2]. Potentially, the gait analysis of the stepping pattern in the mouse may provide a reliable approach for assessing the experimental outcomes and predicting the severity of neurological lesions in the chronic mouse model with relevance to humans with Parkinson's disease. Further investigations are underway in our laboratory to explore the impact of alternative therapies (*i.e.*, pharmacological, exercise, *etc.*) on the gait variability and altered gait certainty seen in the chronic MPTP/P mouse model.

The findings from our investigation also provide insight on the origin of the step-to-step variations seen in gait [3,7,15]. It has been proposed that the general stepping pattern seen in human and animal locomotive patterns is partly governed by a spinal central pattern generator [5]. However, it is not established whether the step-to-step variations could also be the result of the interactions among neuronal groups located in the brain. Since the chronic MPTP/P treatment selectively produces neurological lesions in the nigrostriatal neurons, we propose that the subtle step-to-step variations that are present within the gait pattern are the result of progressive degeneration of the nigrostriatal system in the brain rather than only involving the spinal central pattern generator. These results and further investigations on the step-to-step variations in the chronic mouse model of Parkinson's disease may lead to better understanding of the mechanistic pathways underlying neuronal degeneration and the control of gait pattern.

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