# A novel biomechanical analysis of gait changes in the MPTP mouse model of Parkinson's disease

Werner J Geldenhuys, Tamara Guseman, Ilse S. Pienaar, Dean E Dluzen, Jesse W Young

Parkinson's disease (PD) is an age-associated neurodegenerative disorder hallmarked by a loss of mesencephalic dopaminergic neurons. Accurate recapitulation of the PD movement phenotype in animal models of the disease is critical for understanding disease etiology and developing novel therapeutic treatments. However, most existing behavioral assays currently applied to such animal models fail to adequately detect and subsequently quantify the subtle changes associated with the progressive stages of PD. In this study, we used a video-based analysis system to develop and validate a novel protocol for tracking locomotor performance in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD. We anticipated that 1) treated mice should use slower, shorter, and less frequent strides and 2) that gait deficits should monotonically increase following MPTP administration, as the effects of neurodegeneration become manifest. Video-based biomechanical analyses, utilizing behavioral measures motivated by the comparative biomechanics literature, were used to quantify gait dynamics over a seven-day period following MPTP treatment. Analyses revealed shuffling behaviors consistent with the gait symptoms of advanced PD in humans. Here we also document dramatic gender-based differences in locomotor performance during the progression of the MPTP-induced lesion, despite male and female mice showing similar losses of striatal dopaminergic cells following MPTP administration. Whereas female mice appeared to be protected against gait deficits, males showed multiple changes in gait kinematics, consistent with the loss of locomotor agility and stability. Overall, these data show that the novel video analysis protocol presented here is a robust method capable of detecting subtle changes in gait biomechanics in a mouse model of PD, indicating that this method is a useful means by which to easily and economically screen preclinical therapeutic compounds for protecting against or reversing neuropathology associated with PD neurodegeneration.

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## Introduction

31    Parkinson's disease (PD) is an age-related neurodegenerative disease, where individuals

32    aged older than 60 years of age show increased risk of developing the disorder (Connolly &

33    Lang 2014). A triad of classical motor symptoms is seen in advanced PD patients, consisting of

34    rigidity, akinesia, and tremor (DeLong & Wichmann 2009). These symptoms appear following

35    the loss of at least 80% of the dopaminergic neurons within the Substantia Nigra pars compacta

36    (SNpc) (Hartmann 2004), thereby impairing a patient's ability to perform everyday tasks

37    (Aviles-Olmos et al. 2013). As the disease progresses, co-morbid non-motor symptoms manifest,

38    including cognitive impairments and depression (Lawson et al. 2014; Obeso et al. 2014), which

39    are often resistant to dopamine (DA) replacement therapies (Connolly & Lang 2014). Currently

40    available therapies are mainly aimed at replacing the lost striatal DA content. With dopaminergic

41    cell loss continuing and the side-effects associated with synthetic DA replacement increasing,

42    continuous use of pharmacotherapeutics fails to alter disease progression (Connolly & Lang

43    2014). On the other hand, deep brain stimulation (DBS) therapy shows dramatic improvements

44    in some late-stage PD patients, including improved gait and postural instability following DBS

45    implanted in the pedunculopontine nucleus (PPN), arguing strongly for a case that patients might

46    benefit substantially more, should intervention be initiated at an earlier stage of the disease

47    progression (Mazzone et al. 2014). Related to this, recent work revealed that DBS targeting the

48    subthalamic nucleus (STN) induces vascular remodeling effects, including an upregulation of the

49    vascular endothelial growth factor (VEGF), suggesting that DBS induces plasticity-related

50    effects (Pienaar et al. 2015). Hence, optimization of intervention protocols stand to benefit

51    greatly from a reliable animal model of PD that mimics progressive stages of the disease, in line

52    with the clinical aim of initiating treatment at an earlier stage during progressive PD.

53    Accurate recapitulation of the movement phenotype seen in PD patients in animals is

54    important for the assessment and development of novel therapeutic treatments as well as for

55    providing a tool by which to gain insights into the molecular and cellular mechanisms

56    contributing to the loss of neurons and the concomitant circuit disruptions that characterize

57    human PD. In this regard, sensitive behavioral paradigms are of paramount importance for

58    characterizing existing and newly introduced genetic-based and toxin-induced animal models of

59    neurodegenerative disease (Bury & Pienaar 2013; Pienaar et al. 2012). Unfortunately, many of

60    the behavioral assays applied to animal models fail to adequately detect the subtle changes

61    associated with the different stages of the disease (Antony et al. 2011; Meredith & Rademacher

62    2011). In this study, we used a novel video-based paradigm for analyzing gait and locomotor

63    kinematics to detect the subtle longitudinal changes in locomotor performance occurring in the

64    methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD, and analyzed the

65    results in a gender-specific manner.  Mice and non-human primates systemically injected with

66    MPTP, a mitochondrial neurotoxin, show loss of mesencephalic dopaminergic neurons with

67    concomitant loss of striatal DA content, resulting in motor deficits (Bezard & Przedborski 2011;

68    Pienaar et al. 2012; Schmidt & Ferger 2001). We anticipated that 1) MPTP-treated mice should

69    move at slower speeds and with shorter, less frequent strides, mimicking the bradykinesia shown

70    by PD patients (Fernagut et al. 2002) and that 2) gait deficits should monotonically increase

71    following MPTP administration, as the effects of neurodegeneration become manifest (Klemann

72    et al. 2015). Finally, we also tested for a gender effect in the locomotor response to MPTP

73    treatment, given that previous research has established sex-based differences in the phenotype of

74    both PD patients and rodent models of the disease (Antzoulatos et al. 2010; Gillies et al. 2014;

75    Van Den Eeden et al. 2003).

## Materials and Methods

76

*Study design*

77

78       Research was carried out at Northeast Ohio Medical University (NEOMED), in strict

79    accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals

80    of the National Institutes of Health. All procedures were pre-approved by the NEOMED

81    Institutional Animal Care and Use Committee (NEOMED IACUC Protocol 10-006).

82       Three cohorts of C57BL/6J laboratory mice (n = 4 per group, each consisting of 2 males

83    and 2 females) were used. One male mouse from the first cohort and one female mouse from the

84    second cohort did not show the characteristic striatal DA depletion following MPTP treatment.

85    Additionally, one female mouse from the third cohort lost a significant amount of body weight

86    following MPTP treatment and was removed from the study. Therefore, our final sample

87    consisted of nine mice (five males and four females). On average, male mice weighed more than

88    the female mice (mean female body mass [95% confidence limits]: 23.7 g [23.02 g, 24.32 g],

89    mean male body mass: 29.1 g [27.56 g, 30.66 g]; measured prior to MPTP treatment).

90       The three cohorts were tested in chronological order, such that at the start of data

91    collection, the mice in cohort 1 were 12 weeks of age, those in cohort 2 were 14 weeks of age,

92    and those in cohort 3 were 16 weeks of age. Following a training period lasting from 3-4 days,

93    during which mice were acclimated to the experimental procedure, each animal was evaluated in

94    an initial behavioral assessment to quantify baseline locomotor performance. Following this

95    baseline assessment, mice were treated with a single dose of MPTP (35 mg/kg; i.p.) and then

96    evaluated for seven days of longitudinal locomotor testing, starting at the first day post treatment,

97    but at least 24h after MPTP treatment to bypass some of the most acute phenotypic effects due to

98    the drug's toxicity (e.g., epilepsy-like symptoms: Klemann et al. 2015). In addition to the

99    baseline evaluation, every mouse in the dataset was evaluated at the seventh day after treatment.

100   However, to improve the efficiency of our data collection and reduce the activity burden on the

101   mice, on the remaining days (i.e. days 1-6 following MPTP treatment) experiments were

102   staggered to ensure that we collected data from at least two cohorts of mice per experimental day

103   (i.e. cohort 1 was evaluated on days 1-4, cohort 2 on days 1, 2, 5, 6, and cohort 3 on days 3-7).

104   Mice were then euthanized via cervical dislocation, the striata removed and the DA levels

105   determined using high-pressure liquid chromatography (HPLC), described below.

106         We chose not to include a "sham" control group in our experimental design, but to rather

107   use mixed-model repeated measures statistical analyses (detailed below) to test for the gait-

108   related sequelae of MPTP treatment against each individual's pre-treatment baseline. This design

109   reduced the number of animals required for our study, satisfying ethical concerns relating to

110   reducing the number of animal subjects used for achieving robust statistical results. Our

111   statistical design was combined with an intensive data collection protocol (15-25 locomotor trials

112   collected per day, per experimental animal), enabling us to accurately characterize the efficacy of

113   our new method and obtain statistically significant results, despite a relatively small sample size.

114   *Apparatus and locomotor testing*

115         The mice were made to run along a white wooden trackway (41.75 cm long, 4 cm wide)

116   into a dark box located at the terminal end of the track. The trackway was placed on a laboratory

117   bench top, approximately 1 m off the ground. Mice were filmed with a high-speed digital camera

118   (MotionScope Model PCI 1000s, Redlake MASD Inc., USA) placed overhead to allow for a

119   dorsal view of the mouse running along the trackway. Videos were recorded at 200 frames per

120   second (fps) with a 1/2000 shutter speed. The trackway was illuminated with a 250-watt quartz

121   light (Lowel-Light Manufacturing Inc., USA) to provide adequate depth of field. On either side

122    of the runway, mirrors were placed at 45° angles to the sagittal plane to provide complete views

123    of the footfalls of each limb during locomotion (Fig. 1). We recorded 15-25 strides per mouse

124    per day. All testing took place between 10AM and 2PM, during the active (dark) phase of these

125    nocturnal animals.

126    *Analyses of locomotor performance*

127    The recorded sequences were ported into ProAnalyst motion analysis software (Xcitex

128    Inc., USA) for off-line analyses. We marked the touchdown and lift-off of each forelimb and

129    hindlimb, recognizing touchdown as the first frame in which the limb contacted the runway and

130    lift-off as the first subsequent frame in which the limb was no longer in contact. Individual

131    locomotor strides were identified based on the touchdown of a reference limb (e.g. from a

132    touchdown of the left hindlimb to the next touchdown of the same limb). Only "symmetrical"

133    walking and running strides were included in the final dataset, excluding all high-speed

134    "asymmetrical" bounding and galloping strides (Hildebrand 1965). Strides during which a mouse

135    walked on the side mirrors were excluded from the dataset. Overall, approximately 80% of

136    recorded strides were included in the final dataset.

137    We digitized the two-dimensional position of the tip of the nose and the base of the tail

138    across each pass down the runway. To mitigate digitizing errors and interpolate feature positions

139    across occluded frames, each digitized feature was fit to a quintic smoothing spline function

140    (tolerance: 1 mm$^2$) using MATLAB (version R2014b, MathWorks, Natick, MA, USA) (Walker

141    1998). In order to measure an individual animal's net movement trajectory, we modeled mice as

142    point masses localized at the average static center of mass (COM) position for each gender.

143    Measurements of static COM position in cadaveric male and female mice (N=3 males and N=2

144    females) using the reaction board method (Ozkaya & Nordin 1999; Young 2012) showed some

145 variation between genders, but little variation between individuals within genders (male mean

146 COM position: 41.5% of nose-to-tail base length, coefficient of variation: 6.9%; female mean

147 COM position 45.5% of nose to tail length, coefficient of variation 3.1%). The COM was

148 therefore defined as a point 41.5% along the length of the nose-to-tail base vector in males and

149 45.5% along the length of the nose-to-tail base vector in females.

150 Although our proxy is necessarily a simplification of actual COM position, which can be

151 expected to vary based on both body configuration during locomotion, variation in the effective

152 length of the trunk (i.e. the euclidean distance between the nose and the tail base) was minimal

153 during locomotor strides (average coefficient of variation with 95% confidence bounds, 4.08%

154 [3.822%, 4.337%] in males, 3.81% [3.507%, 4.114%] in females). These data suggest that little

155 lateral flexion of the vertebral column had occurred and indicate that our approach of modeling

156 the trunk as a linear vector should have minimal effect on the accuracy of our COM estimates.

157 Raw data on footfall timings and modeled COM displacements were used to calculate

158 several metrics of locomotor performance. Average locomotor speed (cm/sec) was calculated as

159 the absolute value of mean velocity across the stride. Stride length (cm) was defined as the net

160 distance travelled by the COM during the stride. Stride frequency (Hz) was calculated as the

161 inverse of stride duration. Additionally, we calculated two metrics of overall postural stability.

162 First, based on the timings of limb touchdown and liftoff events, we calculated the percentage of

163 stride duration spent in various support combinations (e.g. supported by one, two, three or four

164 limbs). We then calculated the mean support number using the equation: % single-limb support +

165 (2 × % double-limb support) + (3 × % triple-limb support) + (4 × % quadruple-limb support). A

166 higher mean support number indicates that a greater number of limbs provide support at any one

167 instance during the stride, theoretically conferring greater postural stability to the animal.

168   Additionally, we calculated a "sway" index to quantify mediolateral stability, based on the

169   "straightness" index of Jamon and Clarac (Jamon & Clarac 1998).   The sway index was

170   computed as the ratio of the total horizontal path distance travelled by the COM during the stride

171   and the straight-line distance between the COM coordinates at the beginning and the end of the

172   stride, multiplied by 100. A sway index of 100 indicates a perfectly linear path with higher

173   values indicating increasing amounts of mediolateral sway.

174   *DA content analysis*

175       We measured remaining levels of DA following systemic MPTP treatment, as previously

176   described (Geldenhuys et al. 2014). In brief, mice were euthanized via cervical dislocation, the

177   brain was removed from the skull, and striatal tissue was dissected out then snap frozen in liquid

178   nitrogen. For processing, the striatal tissue was weighed and placed in cold perchloric acid (0.1 N,

179   500 µL, 4 °C). Tissue samples were sonicated and centrifuged. An aliquot was removed to

180   measure DA levels within the bilateral striata across the various cohorts. Tissue samples were

181   evaluated for DA content by means of HPLC coupled with electrochemical detection. Biogenic

182   amines were separated on a Supelco column (Discovery C18, 10 cm × 3 mm × 5 µm). Samples

183   were injected into a 20 µL loop. A degassed isogradient mobile phase consisting of sodium

184   acetate (50 mM), citric acid (27.4 mM), sodium hydroxide (10 mM), sodium octyl sulfate (0.1

185   mM), ethylenediaminetetraacetic acid (EDTA) (0.1 mM) and 5% methanol in filtered deionized

186   water was used for the system. The mobile phase was adjusted to a final pH of 4.5 with the

187   addition of NaOH, with was and filtered (0.45 µm, Millipore filter, USA) prior to use. Standards

188   were diluted in perchloric acid (0.1 N) in increments of 3.1, 6.2, 12.5, 25, 50, 100, 200, and 400

189   pg/20 µL. Samples were analyzed by using the Chromelian 6.8 software program (Dionex, USA).

190   The assay sensitivity (6.2–12.5 pg/20 µL) was determined by observing reliable peaks above

191   baseline noise. Striatal DA values were compared to DA levels in a vehicle-control group of six

192   mice (three males and three females) made up of littermates from the three cohorts of MPTP-

193   treated mice (i.e. one male and one female from each cohort).

194   *Statistical analysis*

195   We used a rank-based Student's *t*-test with the Welch-Satthewaite correction for

196   heteroscedasticity to compare DA loss between genders. This method was recommended by

197   Ruxton (2006) as the most robust method for two-group mean comparisons. We used linear

198   mixed-effects Analyses of Covariance (ANCOVA) to test for gender differences in the

199   longitudinal effects of MPTP toxicity on locomotor performance. The mixed-effects model

200   allowed us to incorporate all relevant fixed factors into our analyses (i.e. gender and days since

201   MPTP treatment), whilst controlling for random variation amongst the mice within each gender

202   group (Batka et al. 2014). All measures were averaged across strides within each experimental

203   day for each mouse, so that the unit of analysis was set as the average response of each mouse on

204   each day of testing. We fitted a full model for each measure, testing for 1) a main effect of

205   gender, 2) a significant association with the number of days since MPTP treatment and 3) an

206   interaction between gender and the number of days since MPTP treatment. A significant

207   interaction indicates that males and females responded differently to MPTP toxicity. In this case,

208   we report separate gender-specific regression slopes (and 95% confidence intervals on those

209   slopes) in order to better illustrate the magnitude and direction of the interaction effect.

210   Additionally, due to the pervasive influence of locomotor speed on most measures of rodent

211   locomotor performance (Batka et al. 2014), in the event of a significant gender-specific

212   regression, we reanalyzed the relationship between the performance variable and the days since

213   MPTP treatment with speed included as a covariate in the model. In these cases, any significant

214  residual association between a performance variable and the days since MPTP treatment

215  indicates that the indicated change in gait mechanics is not simply an after-effect of mice

216  changing their average locomotor speed. All statistical analyses were performed using the R

217  statistical package (R Core Team 2013), including the nlme (Pinheiro et al. 2013) and lsmeans

218  (Lenth 2014) add-on packages.

219  **Results**

220  *Striatal DA levels*

221     Relative to control values, striatal DA levels in MPTP-treated mice decreased by an

222  average of 54.8% amongst treated males and 58.8% amongst treated females (Fig. 2). Although

223  striatal DA values in both genders were significantly lower than those in the vehicle control

224  group (males: $t_{[4]}$=-7.8, $p$=0.001; female: $t_{[3]}$=-7.7, $p$=0.005), loss of striatal DA was statistically

225  similar between genders ($t_{[7]}$=0.48, $p$=0.64).

226  *Locomotor performance*

227     A total of 668 symmetrical strides were analyzed for this study (287 strides from female

228  mice, 381 strides from male mice). A breakdown of the number of strides coded for each

229  experiment day and grouped by gender is provided in Table 1. Variation in the number of valid

230  strides available for analysis on each day accounts for the unequal number of strides across the

231  tabular cells.

232     Mixed-effects analyses of covariance indicated significant gender-by-days-since-MPTP-

233  treatment interaction for all variables ($p \leq 0.029$; results summarized in Table 2). In each case,

234  post-hoc analysis of gender-specific regression slopes indicated that the locomotor performance

235  of males significantly changed following MPTP administration, whereas females showed no

236 significant response to MPTP treatment (i.e. the 95% confidence intervals about the female

237 slopes encompassed zero). Male mice responded to MPTP toxicity by decreasing speed, taking

238 shorter and fewer strides (i.e. decreasing stride length and frequency), increasing the average

239 number of supporting limbs on the ground during the stride (i.e. increasing mean support

240 number) and decreasing mediolateral sway (i.e. lower sway indices) (Table 2; Fig. 3).

241 Because speed has been shown to exert a pervasive influence on other measures of

242 locomotor performance in rodents (Batka et al. 2014), we reassessed the significant relationships

243 between the number of days following MPTP treatment and stride length, stride frequency, mean

244 support number, and sway index in the male mice, whilst controlling for speed using linear

245 mixed-effects multiple regression (results summarized in Table 3). All four variables are

246 significantly associated with speed ($p < 0.001$), suggesting that longitudinal changes in speed

247 may have affected other measures of locomotor performance. Indeed, after controlling for speed,

248 longitudinal decreases in stride length and stride frequency were found to no longer reach

249 statistical significance ($p \geq 0.415$). In contrast, longitudinal changes in mean support number and

250 sway index remained significant even after including speed as a covariate in the model (all $p \leq$

251 0.032).

### Discussion

253 Tests designed to economically and accurately assess gait function in PD animal models

254 are currently lacking (but see Amende et al. 2005). Sensitive assessment tools capable of

255 capturing the different stages of PD in well-established animal models of the human condition

256 remain a challenge to PD researchers interested in screening for and developing treatments and

257 diagnostic markers for translational use (Pienaar et al. 2012). Here we show that videography-

258 based measurements of gait kinematics represent a robust method to assess behavioral deficits in

259  a well-validated mouse model of PD. In PD patients, alterations in the functioning of the

260  nigrostriatal dopaminergic system (NSDA) manifest as changes in sensorimotor functions

261  (DeLong & Wichmann 2009). In mice, PD can be modeled to a remarkably accurate extent with

262  systematic treatment of the relatively selective NSDA neurotoxin, MPTP. A potential drawback

263  of this model is that seeing early changes in the motor behavior of mice when treated with MPTP

264  may be difficult to observe (Blandini & Armentero 2012; Bove & Perier 2012; Morin et al. 2014;

265  Tieu 2011). One possible explanation for this difficulty in detecting early motor changes in mice

266  is that current behavioral assays employed by PD researchers are insensitive to the subtle

267  behavioral changes shown by genetic-based or toxin-induced animal models of human PD

268  (Pienaar et al. 2012). These metrics, which typically include gait measures (e.g. stride length:

269  Fernagut et al. 2002), the pole test (i.e. time required to descend a given pole: Matsuura et al.

270  1997) for assessing bradykinesia, performance on a rotarod spindle for measuring balance, grip

271  strength and motor coordination (Rozas et al. 1997), and a balancing task, assessed by means of

272  a beam walking task (McDermott et al. 1994). However, these tests provide only a gross

273  assessment of sensorimotor function, with little distinction made between the precise functional

274  impairments. Additionally, in many of these assays, rodents are required to perform tasks that are

275  outside of their natural locomotor behavior, making it difficult to see subtle changes in function.

276      Accordingly, marked benefits could ensue with the use of novel approaches and

277  perspectives for the study of motor behavior in animal models of PD. In particular, detailed

278  analyses of gait and balance during normal rodent locomotion promise to reveal subtle changes

279  in motor behavior that may be difficult to detect using common techniques (Wang et al. 2012).

280  Moreover, such analyses have the potential to better model the bradykinesia (slowed

281  movements), festination (short, shuffling steps), postural abnormality, and gait instability that

282    characterizes PD patients. The significance of such an approach is the prospective for identifying

283    specific sensorimotor behavioral markers of NSDA neurodegeneration. Since symptoms

284    associated with PD are not evident until advanced stages of NSDA neurodegeneration, the

285    possibility for identifying behavioral changes at an earlier stage of lesion progression in animal

286    models of PD may permit more accurate screening of novel drugs or stimulatory implants,

287    informing our understanding of the etiology of the human form of the disease and leading to

288    more promising treatments.

289    One approach for performing such sophisticated analyses of motor behavior consists of

290    the biomechanical analysis of gait dynamics. Recently, Wang and others (2012) commented that

291    gait measures specifically focusing on murine models of PD are currently lacking in the literature.

292    The precise, quantitative descriptions of animal movement presented in this study have the

293    potential to identify more subtle behavioral outputs of motor-related neurodegeneration than

294    currently available locomotor assays offer. Moreover, the robust comparative literature that

295    currently exists on the biomechanics of animal quadrupedal gait (Batka et al. 2014; Young 2012)

296    offers a solid methodological and theoretical framework from which to construct and evaluate

297    the analyses used in our research.

298    The use of the video analysis system was able to give significant insights into the gait

299    deficits, which were subtle at times, of MPTP-induced Parkinsonian mice. In male mice, but not

300    in the females, there was a significant change in gait behavior following the loss of striatal DA

301    content (Fig. 3). Using our novel experimental protocol, we were able to observe changes in the

302    gait dynamics of the mice soon after MPTP administration that correlated with the bradykinesia

303    and hyper-rigidity seen in PD patients (Hanakawa et al. 1999). Specifically, following MPTP

304    administration, male mice used slower, shorter and less frequent strides, selected footfall patterns

305  that ensured a greater number of supporting limbs across the stride and decreased mediolateral

306  sway (Fig. 3). The gait deficits seen in the male mice became more pronounced throughout the

307  period following MPTP treatment, indicating a longitudinal decrease in locomotor performance

308  as the neurodegenerative effects of MPTP-toxicity became more pronounced (Klemann et al.

309  2015). Although loss in stride length and frequency appeared to be side effects of the general

310  decrease in locomotor speed, longitudinal changes in mean support number and sway index took

311  place independently of the changes in locomotor speed (Table 3). The longitudinal increase in

312  mean support number suggests that male mice altered their gait in some way to ensure that a

313  greater number of limbs contacted the floor surface throughout the stride, serving as a

314  compensatory effect for promoting greater gait control, to likely be an attempt at increasing

315  locomotor stability. Similarly, the longitudinal decline in the sway index may indicate a

316  decreased ability to cope with challenges to mediolateral stability, as recently demonstrated in

317  human PD patients (Galna et al. 2013). Although it is possible that some of the gait changes

318  observed during the first 1-2 days post-injection could have been associated with an acute

319  inflammatory response to MPTP, subsequent deficits in locomotor performance are best

320  explained as sequelae of neurodegeneration, mimicking the phenotypic processes that occur

321  during human PD (Klemann et al. 2015), mimicking the phenotypic processes that occur during

322  human PD.

323      Interestingly, MPTP-treated female mice showed a dramatic difference in response to the

324  MPTP toxicity. Taking all of the results into account, female mice showed a delayed and

325  diminished response to MPTP on gait function, agreeing with findings that premenopausal

326  women are at lower risk of developing Parkinsonism (Van Den Eeden et al. 2003), and that the

327  clinical phenotype of PD in women often shows a delayed presentation that is reduced in severity

328   when compared to men (Gillies et al. 2014). Our findings are also in agreement with previous

329   studies on the gender bias in MPTP treated mice (Antzoulatos et al. 2010).

330        Although the posthumous processing of striatal tissue indicated similar percent losses of

331   dopaminergic neurons in males and females in response to MPTP, C57BL/6J females typically

332   show substantially greater absolute levels of striatal DA (Dluzen et al. 1996). Accordingly, a

333   critical absolute concentration of DA may remain in females to enable their relatively better gait

334   performance.  It is also likely that the observed gender differences in gait dysfunction are due to

335   compensatory processes unique to females.  Specifically, estradiol has been shown to promote

336   DA turnover, -synthesis, and -release, while simultaneously suppressing reuptake, providing a

337   sex-specific buffering mechanism for preserving behavioral function despite the depletion of

338   dopaminergic populations of neurons (Dluzen & Horstink 2003; Gillies et al. 2014).   While

339   estrogen is capable of producing neuroprotection against MPTP in both male and female

340   C57BL/6J mice (Dluzen et al. 1996), the greater endogenous levels of estrogen along with

341   greater basal striatal DA concentrations of females may contribute to such compensatory

342   processes resulting in a maintenance of female performance in our locomotor task.

**Conclusions**

344        Economical tests designed to accurately assess natural gait function in PD animal models

345   are currently lacking. Here we illustrate the ability of a novel testing protocol using video-based

346   analysis of movement to provide insight into the gait performance of MPTP-treated mice. We

347   were able to show changes in gait function in early stages following MPTP treatment. The male

348   group showed a statistically significant higher propensity towards gait changes than the female

349   mice, suggesting that gait deficits in female MPTP-treated mice might be subtler. Future work

350   should consider carrying out the described gait analyses in a cross-sectional manner, allowing for

351    repeated assays of striatal DA content following MPTP administration to facilitate more precise

352    testing for possible associations between striatal DA depletion and locomotor dysfunction.

353    Overall, the results of our study demonstrate clearly that the novel methodology proposed here

354    has the potential to precisely quantify gait changes in animal models of PD. As such, our

355    measures could be used as output metrics in the initial screening and optimization of compounds

356    and surgical interventions for slowing, or even reversing, disease progression.

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## Supporting Information

467 **Supporting Information**

468     **S1 Data.** Supporting data file. The data from this study are provided in a comma-

469 separated (.CSV) file containing the locomotor measures and striatal dopamine levels for each

470 mouse in the dataset. Note that dopamine levels were evaluated relative to a control value of

471 19,037 obtained by assaying the striatal tissue of six non-treated mice.

472 **Figure Legends**

473 **Figure 1.** Sample frame from high-speed video analyses of mouse locomotion. Video analysis

474 software was used to digitize the two-dimensional position of the nose and tail base during the

475 pass down the trackway. A pair of mirrors angled at 45° to the sagittal plane allowed for

476 capturing touchdown and lift-off events over complete gait cycles.

477

478 **Figure 2.** Measures of striatal DA levels in male and female mice. Each bar represents mean ±

479 S.D. (N females = 4, N males= 5). Asterisks indicate that DA levels in treated males and females

480 were significantly lower than in the vehicle-control group ($p<0.01$ for both genders).

481

482 **Figure 3.** Longitudinal changes in locomotor performance following MPTP administration in

483 male and female mice. Panels show the individual mean values of (a) locomotor speed, (b) stride

484 length, (c) stride frequency, (d) mean support number and (e) sway index at baseline (BL) and at

485 each day following MPTP treatment. Trend lines indicate gender-specific linear mixed-effects

486 regression slopes.

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Tables

1 **TABLES**

2 **Table 1.** Sample sizes of locomotor strides, grouped by gender and MPTP treatment day.

| | | | | MPTP Treatment Day | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Baseline** | **Day 1** | **Day 2** | **Day 3** | **Day 4** | **Day 5** | **Day 6** | **Day 7** |
| **Female** | 56 | 40 | 28 | 23 | 24 | 39 | 35 | 42 |
| **Male** | 68 | 43 | 30 | 29 | 39 | 53 | 56 | 64 |

4  **Table 2.** Analyses of covariance of locomotor performance variables by gender and MPTP
5  treatment day.

| Variable | Interaction Effect[1] | | Slope [95% confidence interval][2] |
|---|---|---|---|
| **Speed** | -1.87 $F_{[1,43]}= 7.93$ $p = \mathbf{0.007}$ | **Female slope:** | 0.616 [-0.373, 1.61] |
| | | **Male slope:** | -1.25 [-2.15, -0.351] |
| **Stride length** | -0.0998 $F_{[1,43]}= 7.69$ $p = \mathbf{0.008}$ | **Female slope:** | 0.0365 [-0.0173, 0.0902] |
| | | **Male slope:** | -0.0633 [-0.112, -0.0145] |
| **Stride frequency** | -0.136 $F_{[1,43]}= 7.05$ $p = \mathbf{0.011}$ | **Female slope:** | 0.0412 [-0.0351, 0.118] |
| | | **Male slope:** | -0.0945 [-0.164, -0.0252] |
| **Mean support number** | 0.0254 $F_{[1,43]}= 5.07$ $p = \mathbf{0.030}$ | **Female slope:** | 0.00355 [-0.0133, 0.0204] |
| | | **Male slope:** | 0.0290 [0.0136, 0.0443] |
| **Sway index** | -0.0539 $F_{[1,43]}= 8.5$ $p = \mathbf{0.006}$ | **Female slope:** | 0.00996 [-0.0176, 0.0375] |
| | | **Male slope:** | -0.0439 [-0.0689, -0.0189] |

8  [1] The interaction effect indicates the average difference in slope between male and female mice
9  (i.e., male slope – female slope), and the significance of this difference relative to the null
10  expectation of zero.  Significant interactions are indicated by a *p*-value in printed in **bold type**.
11
12  [2] These values indicate the gender specific slope of the performance variable against MPTP
13  treatment day.  95% confidence intervals are displayed as [lower confidence bound, upper
14  confidence bound].  Confidence bounds of opposite sign indicate a non-significant slope.

15  **Table 3.** Association between locomotor performance variables and MPTP treatment day,
16  controlling for speed.

| Variable | Partial regression coefficient [95% confidence interval][1] |
|---|---|
| **Stride length** | -0.016 [-0.0554, 0.0237] $p = 0.415$ |
| **Stride frequency** | -0.002 [-0.0254, 0.0209] $p = 0.842$ |
| **Mean support number** | 0.010 [0.0001, 0.0196] $p =$ **0.032** |
| **Sway index** | -0.012 [-0.0214, -0.0018] $p =$ **0.022** |

20  [1] The partial regression coefficients reflect the residual influence of MPTP treatment day on each
21   locomotor performance variable, controlling for the effects of speed.  Significant partial
22   regressions are indicated by a $p$-value in printed in **bold type**.

# 1

Figure 1. Sample frame from high-speed video analyses of mouse locomotion.

Video analysis software was used to digitize the two-dimensional position of the nose and tail base during the pass down the trackway. A pair of mirrors angled at 45° to the sagittal plane allowed for capturing touchdown and lift-off events over complete gait cycles.
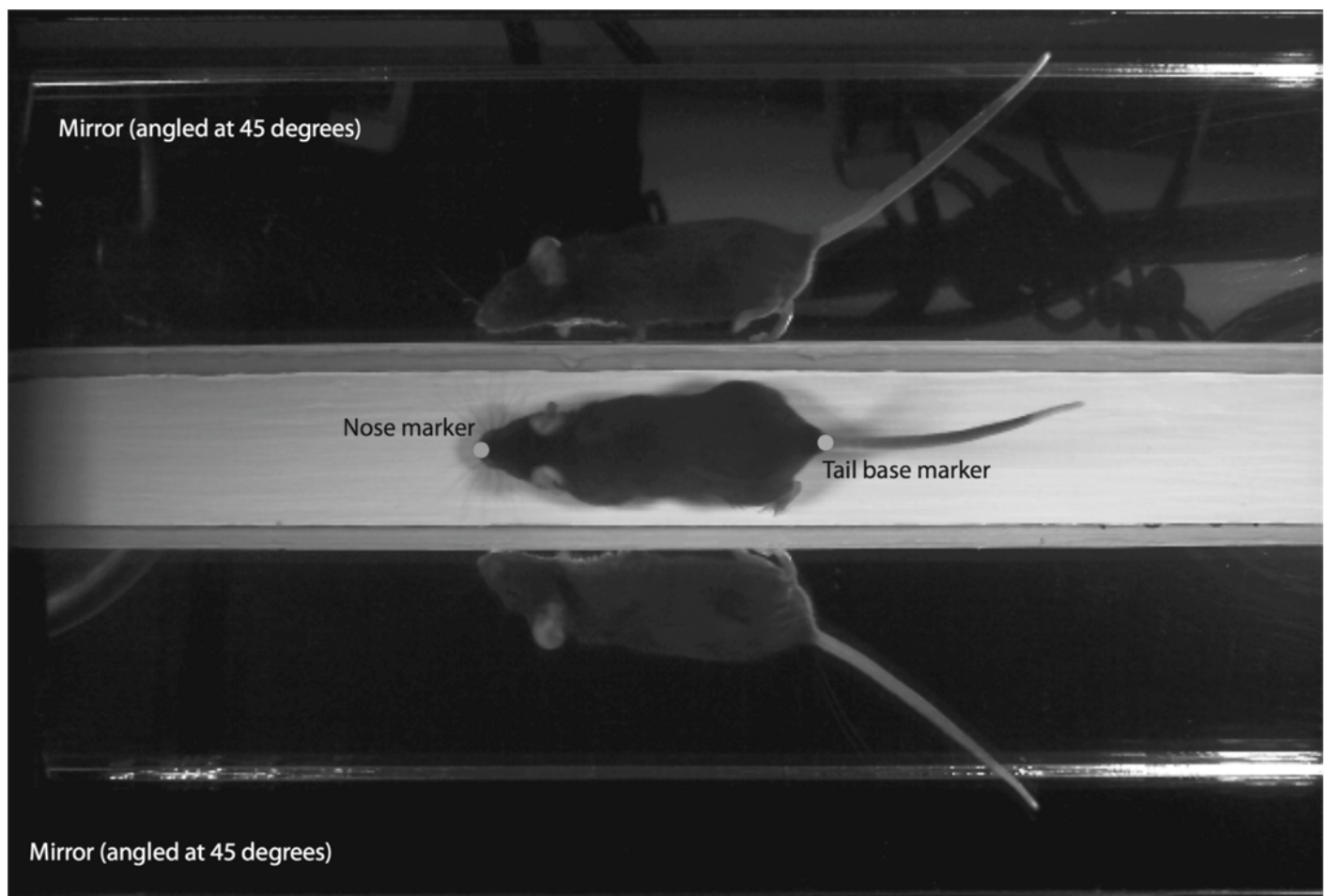
Figure 2. Measures of striatal DA levels in male and female mice.

Each bar represents mean ± S.D. (N females = 4, N males= 5). Asterisks indicate that DA levels in treated males and females were significantly lower than in the vehicle-control group (*p*<0.01 for both genders).
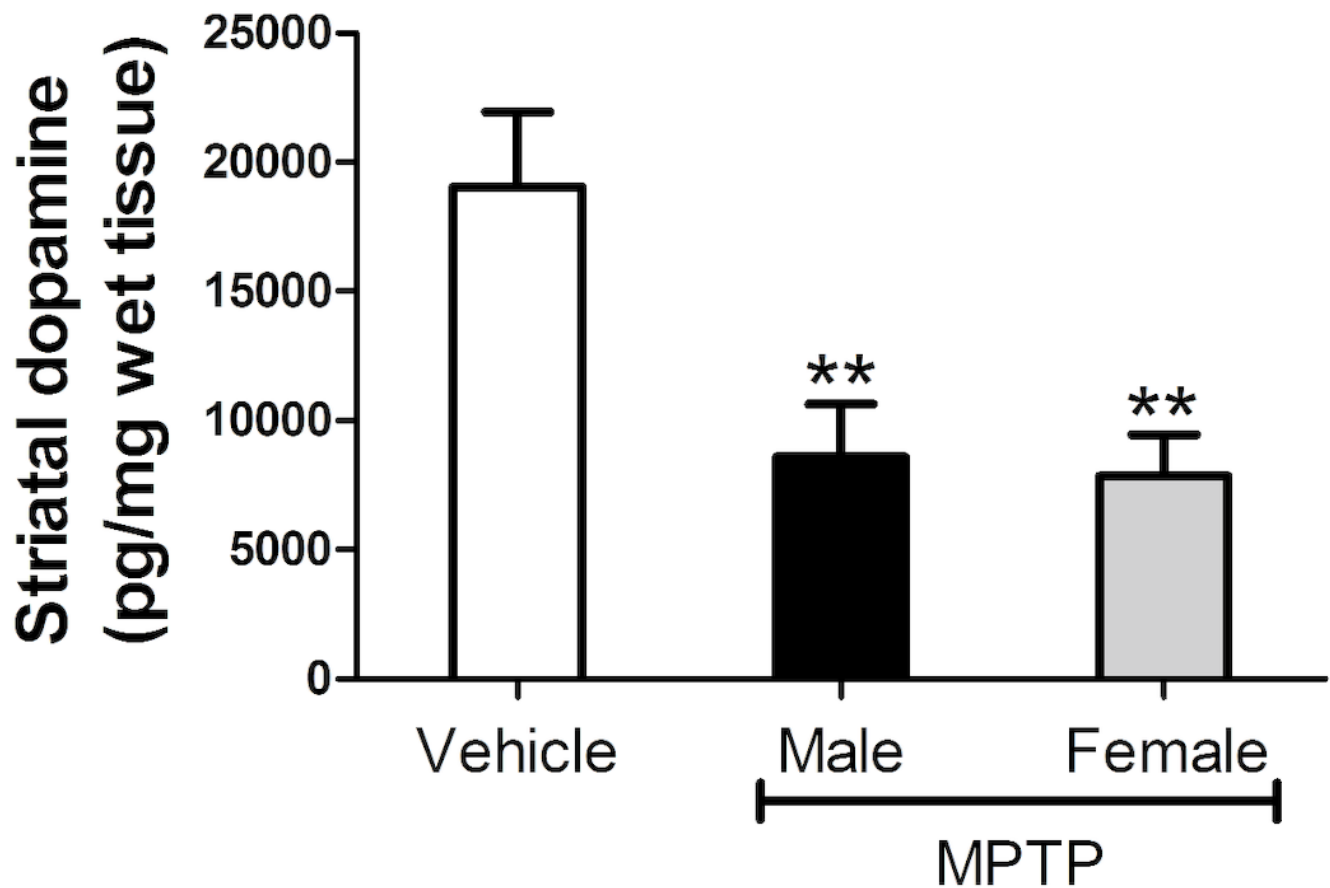
Figure 3. Longitudinal changes in locomotor performance following MPTP administration in male and female mice.

Panels show the individual mean values of (a) speed, (b) stride length, (c) stride frequency, (d) mean support number and (e) sway index at baseline (BL) at each day following MPTP treatment. Trend lines indicate gender-specific linear mixed-effects regression slopes.