Urine Voiding Pattern Analysis in MPTP Mouse Model of Parkinson's Disease

Nipaporn Konthapakdee, Nusaib Sa-ih**[‡], Dania Cheaha[†,‡], Ekkasit Kumarnsit*[‡]*

Abstract

Urinary bladder dysfunction, e.g., urinary frequency, urgency, and incontinence, is one of common non-motor symptoms of patients with Parkinson's disease (PD). 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is widely used to induce Parkinson's disease-like in animal models. However, it remains elusive whether this model reflects urinary bladder dysfunction in PD patients. This study aims to investigate the urine voiding pattern of MPTP-induced Parkinson's disease-like in mice using spontaneous voiding spot analysis (VSA). Male ICR mice (8 weeks old) were injected with MPTP (10 mg/kg in normal saline, i.p.) once a day for 5 consecutive days, while the control group received normal saline. Voiding pattern was investigated at day 21 after the last treatment. Filter paper and metal wire mesh were placed in the cages to determine urine pattern of the animals for 4 hours with free access to food and water. Thereafter, the urine-stained filter paper was visualized under UV light and imaged. Urine spot number, urine volume, number of small and large urine spots, percentage of volume in the center and corner were quantified. MPTP-treated mice had a significant increase in total urine spot number (control, 8.20 ± 3.0, *vs* MPTP, 20.40 ± 3.73, $P < 0.05$, Student's unpaired *t*-test; n = 5 in each group) and number of small spots compared to control (control, 4.60 ± 2.62 *vs* MPTP, 14.80 ± 3.09, $P < 0.05$, Student's unpaired *t*-test; n = 5 each). There was no significant difference in total urine volume, number of large urine spots, and percentage volume in the center and corner of the cages. Our findings suggest that MPTP-induced Parkinsonian-like symptoms in mice could potentially be used as a model to investigate bladder dysfunction in Parkinson's disease.

J Physiol Biomed Sci. 2019; 32(1): 19-24

Keywords: *MPTP, Parkinson's disease, urinary incontinence, voiding behavior, voiding spot analysis (VSA)*

Introduction

Parkinson's disease (PD) is a motor disorder characterized by neurodegeneration of dopaminergic neurons in the substantial nigra par compacta (SNc) and the presence of Lewy bodies in the brain region.[1,2,3] Patients suffer from motor symptoms such as tremor, dyskinesia, and rigidity. Apart from motor function impairment, urinary bladder dysregulation, e.g., urinary frequency, urinary incontinence, and nocturia are common in PD patients.[4,5]

Micturition is a complex function which is partly controlled as a sacral autonomic reflex. In addition, it also depends on the regulation center in the pons which is influenced by the higher brain areas, i.e., cerebral cortex, basal ganglia, thalamic nuclei and the anterior vermis of the cerebellum.[6,7] The micturition reflex is under influence of dopaminergic signaling via inhibitory D1 dopaminergic and excitatory D2 dopaminergic receptors.[7,8] In a physiological condition, the net effect of dopaminergic signaling is inhibitory to micturition via D1 receptor activation. Loss of dopaminergic neurons in SNc in PD leads to a loss of a suppressive effect on the micturition control and results in overactive bladder symptoms, e.g, urinary incontinence, frequency, and urgency.[9,10]

Previous studies reported that dopaminergic neuron lesion causing urinary bladder dysfunctions. Rats with 6-hydroxydopamine (6-OHDA) induced-dopaminergic neuron lesions in the nitro-striatal dopaminergic pathway exhibited bladder hyper-reflexia.[11] Isolated detrusor muscles from marmoset-treated with MPTP showed an increase in frequency and amplitude of contraction.[12] In addition, dopaminergic receptor D1 agonist prevents bladder hyper-activity in MPTP-induced Parkinson's disease-like in marmosets.[13]

MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, is one of the most common neurotoxic model to induce Parkinsonism in animal models. MPTP specifically damages the nigrostriatal dopaminergic neurons with a profound decrease of dopamine level in the striatum and SNc.[14,15] MPTP is a lipophilic molecule which is taken up into the brain by astrocytes and metabolized into MPP^+. MPP^+ further inhibits mitochondrial respiratory complex I and results in neuronal death.[16,17] MPTP injection (30

*Department of Physiology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand;
**Graduate Program in Physiology, Department of Physiology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand;
[†]Department of Biology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand;
[‡]Research Unit for EEG Biomarkers of Neuronal diseases, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, 90112, Thailand.

Corresponding author: Ekkasit Kumarnsit, PhD
E-mail: ekkasit.k@psu.ac.th

Received 27 December 2018, Accepted 29 June 2019

mg/kg) causes a loss of DA neurons greater than 80% after 1 day and a loss of greater than 40% after 30 days in striatal area in mice. In addition, subchronic treatment with a daily injection of MPTP (25 mg/kg) for 5-10 days leads to 53% loss of dopaminergic levels in striatal area in 30 days post-treatment and 24-40% of dopaminergic neuron lesion in SNc.[18] However, whether this MPTP-induced Parkinsonism in animal model could reflect urinary bladder dysfunction in PD is still unclear.

Voiding spot analysis (VSA) is the simplest and non-invasive method to evaluate urinary bladder function via determination of urine marking on filter paper. The urine stained filter papers are imaged by autofluorescence emitted from the urine under ultraviolet light.[19,20] This method is widely used to physiologically investigate the urinary bladder function during awake conditions.[21,22] Therefore, the present study aims to investigate urine voiding pattern in MPTP-induced PD in mice using voiding spot analysis.

Materials and Methods

Animals

Male ICR mice (25-45 g) were obtained from National Laboratory Animal Center, Mahidol University, Bangkok, Thailand. All procedures were conducted with the approval of the Animals Ethical Committee of Prince of Songkla University. All animals were maintained individually in standard cages of the Laboratory Animal Facility Unit, Faculty of Science, Prince of Songkla University, Thailand. All mice were maintained in a room with 22 ± 2 °C and 12:12 hours light:dark condition. The animals could freely access standard chow (S.W.T; Thailand) and water. The animals were allowed to acclimatize for at least 7 days ahead of the experiment.

MPTP treatment

The animals were divided into control and MPTP groups. Mice in the MPTP group were injected intraperitoneally with 10 mg/kg body weight MPTP (Sigma M0896) for 5 consecutive days to induce Parkinson's disease-like symptoms. Control animals received 0.9% normal saline. The animals' body weight was monitored daily.

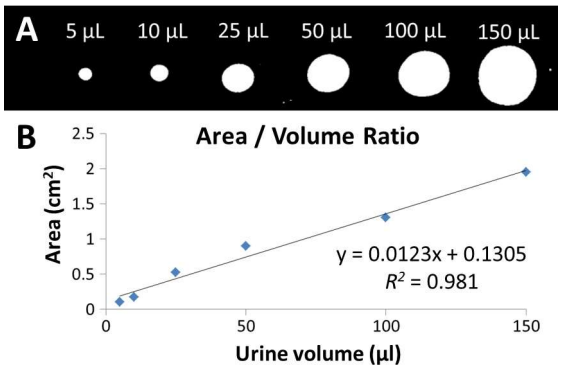

Figure 1 Volumetric calibration of voiding spot analysis: (*A*) Image of mouse urine with various concentrations; (*B*) scatter plot of calibration graph showing a correlation between urine volume and spot area.

Voiding spot analysis (VSA)

Urine pattern of the animals was studied after 21 days of the last MPTP or normal saline injection. Twenty-four hours before testing, mice were allowed to habituate with a metal wire mesh (15 × 26.5 cm^2, with grid spacing 1.2 × 1.0 cm^2) fitted in the standard mouse cage for a 4-hour period between 1 and and 5 pm. On the examination day, filter paper (catalog no. 1003-917, Whatman®) was placed on the bedding material in the standard cage and the metal wire mesh was placed 1.5 cm over the filter paper. All mice were given access to food and water during the experimental period. Thereafter, the filter paper was dried for 24 hours and photographed under the UV light (BioSpectrum® Imaging system, UVP, Upland, California, USA). Urine stained filter was analyzed using Void Whizzard software, version 1.3.[23]

Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM) and analyzed by Student's *t*-test using GraphPad Prism 6 (GraphPad Software, San Diego, California, USA).

Results

There was no significant difference in body weight between control and MPTP groups (control, 43.92 ± 2.46, *vs* MPTP, 44.12 ± 0.25 g). To investigate a correlation between urine volume and urine voiding spot area, a mouse urine calibration curve was established.

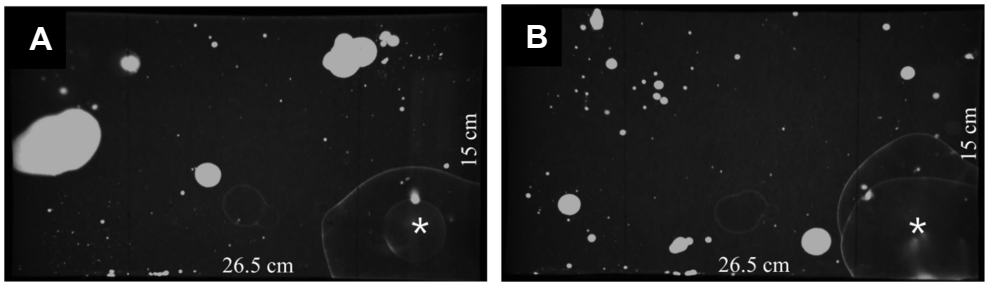

Figure 2 Voiding pattern of control and MPTP-treated mice groups. (*A*) Marking pattern of control mice showed a higher density of large urine spots and non-circular marking compared to MPTP-treated group (*B*); *indicated water dropping from water bottle.

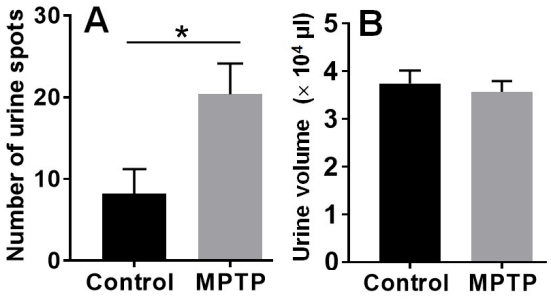

Figure 3 Urine spot number of MPTP-treated mice and control group. (*A*) Urine spot number was significantly increased in MPTP-treated animals compared to control. (*B*) Total urine volume was unchanged (**P* < 0.05, Student's unpaired *t*-test; n=5, each group).

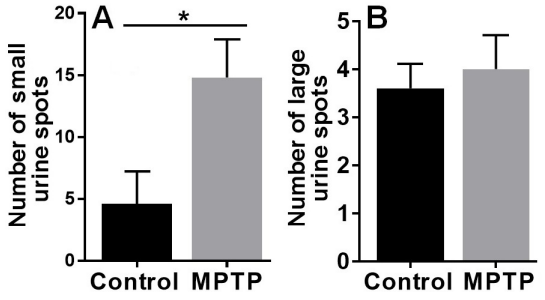

Figure 4 Small and large urine spot number of MPTP-treated mice and control group. (*A*) Small urine spot (< 0.2 cm^2) number was significantly increased in MPTP-treated animals compared to control. (B) Large urine spot (> 0.2 cm^2) was unchanged (**P* < 0.05, Student's unpaired *t*-test; n = 5 in each group).

Different mouse urine volumes (5, 10, 25, 50, 100, and 150 µl) were pipetted on filter paper and the correlation between area and urine volume was determined. We found a strong correlation between urine spot area and urine volume, with $R^2 = 0.981$ (Figure 1).

Representative images of urine-stained filter paper from control mice and MPTP-treated mice are shown in Figure 2. Urination pattern of control mice showed larger markings compared with those of MPTP-treated mice. In addition, MPTP-treated mice had a significant increase in total urine spot number (control, 8.20 ± 3.0, *vs* MPTP, 20.40 ± 3.73, $P < 0.05$, Student's unpaired *t*-test; n = 5 in each group). However, there was no significant difference in total urine volume (Figure 3). The number of small urine spots (< 0.2 cm^2) was higher in MPTP compared to control groups (control, 4.60 ± 2.62, *vs* MPTP, 14.80 ± 3.09, $P < 0.05$, Student's unpaired t-test; n = 5 each; Figure 4A). There was no significant difference in total urine volume and the number of large urine spots (>0.2 cm^2) (Figure 4B), number of urine spot in the center and corner area (Figure 5). These results suggest that MPTP-treated mice may exhibit urinary bladder dysfunction, i.e., urinary incontinence, frequency, and urgency.

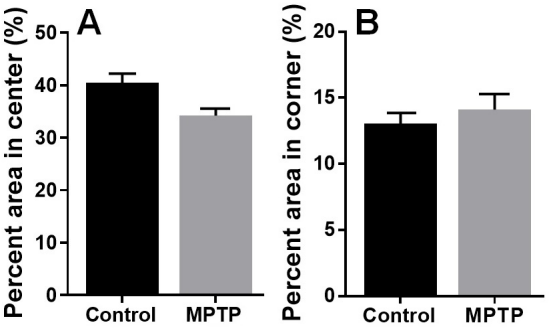

Figure 5 Voiding area in MPTP-treated mice and control group. (*A*) Percent urine area stained in the center and (*B*) corners of filter papers of control group compared to MPTP group (Student's unpaired *t*-test; n = 5 in each group).

Discussion

The present study aims to investigate voiding pattern in MPTP-induced Parkinsonism in mice. We found a clear difference in the pattern of urine spots between control and MPTP-treated animals. Urine pattern of control mice showed non-circular marking with larger volume/spot size and preferential voiding in the corner zone, which is considered normal in mice.[24] This pattern was different from that observed in MPTP-treated group with more circular and smaller urine spots. Interestingly, MPTP-treated mice showed an increase in the number of small urine spots. However, there was no significant difference in total urine volume between control and treated animals. These data suggest that MPTP-treated mice showed signs of urinary urgency, frequency, and incontinence at 21 days after MPTP induction.

We induced Parkinson's disease-like symptoms by using neurotoxin MPTP (10 mg/kg) injection for 5 consecutive days. Even though there was no histological confirmation of dopaminergic neuron lesions in the brain, a previous study reported dopaminergic lesions with 10 mg/kg MPTP, and the total dose per mouse of 40 mg/kg led to a 50% reduction of neurons in SNc.[25] This is in line with another study in which Parkinsonism in rats were induced with 6-OHDA treatments showing detrusor overactivity at day 14 post-injection and the bladder dysfunction persisted for 28 days.[26]

A previous study in isolated detrusor muscle strip of MPTP-treated marmoset showed that there was an increase in amplitude and frequency of contraction. This bladder overactivity sign may be derived from a central dopaminergic lesion leading to a change in neural signaling at the level of presynaptic transmission between nerve and detrusor muscle.[12] In physiological condition, dopamine from substantial nigra par compacta activates D1-GABAnergic direct pathway, which exerts an inhibitory action to micturition.[7,27] Loss of dopaminergic neuron in PD patients may lead to detrusor overactivity, i.e., urinary incontinence and frequency.[10,28] However, future studies are still required to fully validate this hypothesis and the mechanism involved in bladder dysregulation of MPTP-induced Parkinson-like model.

Urinary bladder dysregulation has been reported in transgenic multiple system atrophy (MSA) mice, animals with motor impairments.[29] The urinary bladder dysregulation in MSA is associated with the degeneration of nigral dopaminergic pathways and nondopaminergic areas, including the pontine micturition center, periaqueductal gray, locus coeruleus, cerebellar Purkinje cells, dorsal motor nucleus of the vagus, intermediolateral columns of the spinal cord, and Onuf's nucleus.[30] Functional cystometry revealed the presence of lower urinary bladder dysfunction, i.e., detrusor hypertrophy, in MSA animals, including urinary frequency, urgency, incontinence, and incomplete bladder emptying, resulting in an increased post-void residual volume.[31]

The current study showed that MPTP-induced Parkinsonism related to urinary bladder at 21 days after the induction. An investigation of intestinal function in mice treated with 10 mg/kg MPTP, with the total dose of 40 mg/kg, showed decreased colonic motility reflected via reduced stool frequency. This impaired intestinal function is correlated with decreased neural population in the enteric plexus and increased pro-inflammatory markers, i.e., TNF and iNOS, in the intestinal wall at 18 days post-MPTP injection.[32] However, a previous study has reported that non-motor symptoms are possibly found in all phases even at an early stage of Parkinson's disease.[33]

We think an increase in urination frequency may not be a direct result of MPTP injection. Previously, tissue accumulation levels of MPTP and MPP^+ following either subcutaneous or oral administration of MPTP in mice were examined. Twenty-four hours following subcutaneous injection of 90 mg/kg MPTP, the MPTP and MPP^+concentrations in kidneys were < 20 μg/g.[34] Thus, it is unlikely that an increase in urination frequency observed in this study was a direct effect of MPTP on urinary bladder tissue. Besides, the urine voiding pattern was determined at day 21 after the last MPTP injection, when MPTP would have possibly been cleared from the body systemic circulation.

Our study determined bladder function using voiding spot analysis which is one of the simplest methods to evaluate urinary bladder function in awake animals. Compared to other urinary bladder function test,e.g., cystometry, which is more invasive as it requires an operation, the voiding spot analysis rarely affects the animals' physiological condition since they are allowed to stay in their cage during the test. Voiding spot analysis is a semiquantitative method used to analyze urine volume according to urine spot area on the filter papers. This technique is validated by generating a standard calibration curve of urine volume and area on filter papers. We counted the urine spot area of < 0.2 cm^2 as a small spot or a small urine volume and urine spot area of > 0.2 cm^2 a large spot or a big urine volume as previously described.[21] In addition to urine volume, voiding spot analysis also determines the area of voiding e.g., center and corner areas which could also relate urinary bladder dysfunction with other symptoms as observed in stress-related behavior.[35]

Environmental factors, e.g., housing environment, water bottle location, and cage types, could affect voiding behavior and pattern. A previous study reported that changing the water bottle location could reduce the total void. Using a standard cage seems to provoke more urine volume than using a metabolic cage. Water deprivation during 4 hours did not result in any significant difference in voiding pattern and volume.[20] We minimized the variation from these factors since the tests were performed in their cages with free access to water and food. Moreover, results of voiding spot analysis possibly vary with sex and mouse strain. CAST/EiJ male mice had significantly greater urine spot number compared with female mice. The gender difference has not been found in some other mouse strains (129S1/SvImJ, C57BL/6J and NOD/ShiLtJ).[36] Therefore, voiding spot analysis should be used with a consideration of these factors that may influence result and interpretation from VSA. Hence, the voiding pattern obtained from the VSA method should be combined with another functional method e.g., cystometry which would yield a deeper understanding of urinary bladder dysfunction in PD. One major draw-back of VSA is that when a second or third urination is superimposed on to the first voiding area. It might cause mistakes in counting urine spot numbers. Nevertheless, differences in the density of urine spot between treatments were clear.

Conclusion

This study is the first to report that MPTP-treated mice exhibited an increase in urination function at day 21 post lesion. This suggests that MPTP-induced Parkinson's disease-like symptoms may also be used as a potential tool to investigate urinary bladder symptoms in PD. However, future research is required to further investigate changing bladder function in a different timeline of MPTP treatment and need to be confirmed with other methods to better understand the bladder pathology involved in this animal model.

Acknowledgments

This research project was supported by a grant from thesis research scholarship and research assistant scholarship provided to Miss Nisaib Sa-ih from Graduate School, Prince of Songkla University, Thailand. We would like to thank Dr.Wanisa Salaemae for providing some filter papers and Department of Biochemistry, Faculty of Science, Prince of Songkla University, for a kind support to use BioSprectum® Imaging system.

Conflict of Interest

None to declare.

References

1. Pakkenberg B, Moller A, Gundersen HJ, Dam AM, Pakkenberg H. The absolute number of nerve cells in substantia nigra in normal subjects and in patients with Parkinsons disease estimated with an unbiased stereological method. J Neurol Neurosurg Psychiatry. 1991; 54: 30-3
2. Wolman L, Roy S. The substantia nigra in Parkinsonism. J Clin Pathol. 1969; 22: 507-8.
3. Parkinson J. An essay on the shaking palsy. J Neuropsychiatry Clin Neurosci. 2002; 14: 223-36.
4. Araki I, Kitahara M, Oida T, Kuno S. Voiding dysfunction and Parkinsons disease: Urodynamic abnormalities and urinary symptoms. J Urol. 2000; 164: 1640-3.
5. Sakakibara R, Shinotoh H, Uchiyama T, Sakuma M, Kashiwado M, Yoshiyama M, et al. Questionnaire-based assessment of pelvic organ dysfunction in Parkinsons disease. Auton Neurosci. 2001; 92:76-85.
6. Kavia RBC, Dasgupta R, Fowler CJ. Functional imaging and the central control of the bladder. The J Comp Neurol. 2005; 493: 27-32.
7. de Groat WC. Integrative control of the lower urinary tract: Preclinical perspective. Br J Pharmacol. 2006; 147 Suppl 2: S25-40
8. Yoshimura N, Mizuta E, Yoshida O, Kuno S. Therapeutic effects of dopamine D1/D2 receptor agonists on detrusor hyperreflexia in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned parkinsonian cynomolgus monkeys. J Pharmacol Exp Ther. 1998; 286: 228-33.
9. Blackett H, Walker R, Wood B. Urinary dysfunction in Parkinsons disease: A review. Parkinsonism Relat Disord. 2009; 15: 81-7.
10. Sakakibara R, Tateno F, Kishi M, Tsuyuzaki Y, Uchiyama T, Yamamoto T. Pathophysiology of bladder dysfunction in Parkinsons disease. Neurobiol Dis. 2012; 46: 565-71.
11. Yoshimura N, Kuno S, Chancellor MB, de Groat WC, Seki S. Dopaminergic mechanisms underlying bladder hyperactivity in rats with a unilateral 6-hydroxydopamine (6-OHDA) lesion of the nigrostriatal pathway. Br J Pharmacol. 2003; 139: 1425-32.
12. Pritchard S, Jackson MJ, Hikima A, Lione L, Benham CD, Chaudhuri KR, et al. Altered detrusor contractility in MPTP-treated common marmosets with bladder hyperreflexia. Plos One. 2017; 12: 1-19
13. Yoshimura N, Mizuta E, Kuno S, Sasa M, Yoshida O. The dopamine D1 receptor agonist SKF 38393 suppresses detrusor hyperreflexia in the monkey with parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Neuropharmacol. 1993; 32: 315-21.
14. Dauer W, Przedborski S. Parkinson's disease: Mechanisms and models. Neuron. 2003; 39: 889-909.
15. Langston JW, Forno LS, Rebert CS, Irwin I. Selective nigral toxicity after systemic administration of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyrine (MPTP) in the squirrel monkey. Brain Res. 1984; 292: 390-4.
16. Nicklas WJ, Vyas I, Heikkila RE. Inhibition of NADH-linked oxidation in brain mitochondria by 1-methyl-4-phenyl-pyridine, a metabolite of the neurotoxin, 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine. Life Sci. 1985; 36: 2503-8.
17. Przedborski S, Jackson-Lewis V. Mechanisms of MPTP toxicity. Mov Disord. 1998; 13: 35-8.
18. Petroske E, Meredith G, Callen S, Totterdell S, Lau Y-S. Mouse model of Parkinsonism: a comparison between subacute MPTP and chronic MPTP/probenecid treatment. Neurosci. 2001; 106: 589-601.
19. Arakawa H, Blanchard DC, Arakawa K, Dunlap C, Blanchard RJ. Scent marking behavior as an odorant communication in mice. Neurosci Biobehav Rev. 2008; 32: 1236-48.
20. Chen H, Zhang L, Hill WG, Yu W. Evaluating the voiding spot assay in mice: A simple method with complex environmental interactions. Am J Physiol Renal Physiol. 2017; 313: F1274-80.
21. Birder L, Nakamura Y, Kiss S, Nealen M, Barrick S, Kanai A, et al. Altered urinary bladder function in mice lacking the vanilloid receptor TRPV1. Nat Neurosci. 2002; 5: 856-60.
22. Streng T, Santti R, Talo A. Similarities and differences in female and male rat voiding. Neurourol Urodyn. 2002; 21: 136-41.
23. Wegner KA, Abler LL, Oakes SR, Mehta GS, Ritter KE, Hill WG, Zwaans BM, Lamb LE, Wang Z, Bjorling DE, Ricke WA, Macoska J, Marker PC, Southard-Smith EM, Eliceiri KW, Vezina CM. Void spot assay procedural optimization and software for rapid and objective quantification of rodent voiding function, including overlapping urine spots. Am J Physiol Renal Physiol. 2018;315: F1067-80.
24. Gevaert T, Vriens J, Segal A, Everaerts W, Roskams T, Talavera K, et al. Deletion of the transient receptor potential cation channel TRPV4 impairs murine bladder voiding. J Clin Invest. 2007; 117: 3453-62.
25. Hung LW, Villemagne VL, Cheng L, Sherratt NA, Ayton S, White AR, et al. The hypoxia imaging agent Cu II (atsm) is neuroprotective and improves motor and cognitive functions in multiple animal models of Parkinson's disease. J Exp Med. 2012; 209: 837-54.
26. Soler R, Füllhase C, Santos C, Andersson K-E. Development of bladder dysfunction in a rat model of dopaminergic brain lesion. Neurourol Urodyn. 2010; 30: 188-93.
27. Seki S, Igawa Y, Kaidoh K, Ishizuka O, Nishizawa O, Andersson K-E. Role of dopamine D1 and D2 receptors in the micturition reflex in conscious rats. Neurourol Urodyn. 2000; 20: 105-13.
28. Sakakibara R, Nakazawa K, Uchiyama T, Yoshiyama M, Yamanishi T, Hattori T. Micturition-related electrophysiological properties in the substantia nigra pars compacta and the ventral tegmental area in cats. Auton Neurosci. 2002; 102: 30–8.
29. Stefanova N, Bücke P, Duerr S, Wenning GK. Multiple system atrophy: an update. Lancet Neurol. 2009; 8: 1172-8.
30. Winge K, Fowler CJ. Bladder dysfunction in Parkinsonism: mechanisms, prevalence, symptoms, and management. Mov Disord. 2006; 21: 737-45.
31. Boudes M, Uvin P, Pinto S, Voets T, Fowler CJ, Wenning GK, et al. Bladder dysfunction in a

transgenic mouse model of multiple system atrophy. Mov Disord. 2013; 28: 347-55.
32. Ellett LJ, Hung LW, Munckton R, Sherratt NA, Culvenor J, Grubman A, et al. Restoration of intestinal function in an MPTP model of Parkinson's disease. Sci Rep. 2016; 6: 1–11.
33. Gokcal E, Gur VE, Selvitop R, Yildiz GB, Asil T. Motor and non-motor symptoms in Parkinson's disease: Effects on quality of life. Noro Psikiyatr Ars. 2017; 54: 143-8.
34. Fuller RW, Hemrick-Luecke SK. Tissue concentrations of MPTP and MPP^+ after administration of lethal and sublethal doses of MPTP to mice. Toxicol Lett. 1990; 54: 253-62.
35. Biallosterski BT, Prickaerts J, Rahnama'I MS, Wachter SD, Koeveringe GAV, Meriaux C. Changes in voiding behavior in a mouse model of Alzheimer's disease. Front Aging Neurosci. 2015; 7: 1-7.
36. Bjorling DE, Wang Z, Vezina CM, Ricke WA, Keil KP, Yu W, et al. Evaluation of voiding assays in mice: Impact of genetic strains and sex. Am J Physiol Renal Physiol. 2015; 308: F1369-78.