Heliyon 6 (2020) e05771

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Gastrointestinal and metabolic function in the MPTP-treated macaque model of Parkinson's disease

Anna Delamarre [a,b,1], Cliona MacSweeney [c,1], Rie Suzuki [c], Alastair JH. Brown [c], Qin Li [d], Elsa Y. Pioli [d,1], Erwan Bezard [a,b,d,*,1]

[a] Université de Bordeaux, Institut des Maladies Neurodégénératives, Bordeaux, 33000, France
[b] Centre National de la Recherche Scientifique, Institut des Maladies Neurodégénératives, Bordeaux, 33000, France
[c] Sosei Heptares, Cambridge, CB21 6DG, United Kingdom
[d] Motac Neuroscience, Macclesfield, SK10 4TF, United Kingdom

ARTICLE INFO

Keywords:
Gastroparesis
Intestinal permeability
Insulin resistance
Acetaminophen
FITC-dextran

ABSTRACT

Background: Gastrointestinal (GI) and metabolic function are frequently altered in Parkinson's disease (PD). Although enteric nervous system anatomopathological alterations have previously been reported in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) monkey model of PD, the resulting gastric emptying and intestinal permeability functional parameters are unknown. The current exploratory study was, thus, designed to investigate these GI functional factors and insulin resistance in the MPTP-treated monkey.

Methods: Eight rhesus macaque monkeys (4 controls and 4 MPTP-treated) received the oral acetaminophen absorption test to measure gastric emptying, the oral FITC-dextran absorption test to investigate intestinal permeability, and the intravenous glucose tolerance test to assess insulin resistance. Constipation was evaluated using the Bristol stool scale.

Results: None of the tests, acetaminophen absorption, FITC-dextran absorption or glucose tolerance, showed a difference between control and MPTP-treated monkeys. MPTP-treated monkeys did present signs of transit acceleration.

Conclusion: While the MPTP monkey model reliably displays motor and certain non-motor symptoms of PD, the current study did not demonstrate the GI symptoms associated with PD.

1. Introduction

Parkinson's disease (PD) has long been characterised by the classical motor features of parkinsonism associated with Lewy bodies and loss of dopaminergic neurons in the substantia nigra. However, PD symptomatology is now recognised as heterogeneous, with clinically significant non-motor features such as gastrointestinal (GI) dysfunction, that are thought to start years before the onset of classic parkinsonism (Kalia and Lang 2015). GI alterations affect every part of the GI tract, with dysphagia, drooling, gastroparesis, small intestine bacterial outgrowth and constipation constituting the major GI-related symptoms (Fasano et al., 2015; Pfeiffer 2018). Constipation in particular is associated with risk of developing PD in the future (Abbott et al., 2001). From a physiological standpoint, an impairment of intestinal permeability is also reported in PD (Forsyth et al., 2011; Davies et al., 1996). Interestingly, this "leaky gut" may facilitate access of environmental and microbiota toxins to mucosal compartment and promote inflammatory responses, raising the possibility that a "leaky gut" could play a role in α-synuclein accumulation in the enteric nervous system (ENS) (Forsyth et al., 2011; van IJzendoorn and Derkinderen 2019).

A major roadblock in further understanding the GI dysfunction pathophysiology, and hence future therapeutic developments, is the lack of validated animal models mimicking GI phenomenology in PD. Limited investigations in transgenic rodents (McDowell and Chesselet 2012; Greene 2011) as well as scarce neuropathological reports of enteric nervous system alterations in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) monkey model of PD (Chaumette et al., 2009) suggest that constipation and enteric nervous system neuropathology (MPTP monkey) might be, at least in part, mimicked.

* Corresponding author.
E-mail address: erwan.bezard@u-bordeaux.fr (E. Bezard).
[1] Equal contribution.

https://doi.org/10.1016/j.heliyon.2020.e05771
Received 20 July 2020; Received in revised form 7 October 2020; Accepted 15 December 2020

Facing the lack of evidence, we thus investigated GI and metabolic functions in the MPTP-treated monkey model of PD. This exploratory study, performed at a single time-point, used the Bristol stool scale to evaluate constipation, the oral acetaminophen absorption test to measure gastric emptying, oral Fluorescein Isothiocyanate (FITC)-dextran absorption test for intestinal permeability, and the intravenous glucose tolerance test to assess insulin resistance.

2. Materiel & methods

2.1. MPTP monkeys

The study was performed in accordance with the European Union directive (2010/63/EU) on the protection of animals used for scientific purposes with local approval by the Institutional Animal Care and Use Committee (IACUC) of the Institute of Laboratory Animal Science (ILAS) at the Chinese Academy of Medical Science (CAMS, Beijing, PRC). Eight male rhesus macaques (*Macaca mulatta*, captive bred animals form Xieerxin Laboratory Co. Ltd., Beijing, China; 5.5 ± 0.2 kg) were housed in an AAALAC-accredited facility. Environmental enrichment was provided in the home cages. Drinking water was available *ad libitum*. Food pellets were given 3 times a day and fruit twice daily.

The MPTP intoxication protocol and the clinical assessments were conducted as previously published (Ahmed et al., 2010; Aubert et al., 2005; Bezard et al. 2001, 2003; Porras et al., 2012; Shen et al., 2015; Urs et al., 2015), giving rise to animals displaying a very large nigrostriatal lesion, i.e. above 80% of dopamine neurons in the substantia nigra (Bezard et al., 2001) and over 90% decrease in striatal dopamine content (Engeln et al., 2015). Animals were rendered parkinsonian with MPTP-hydrochloride (0.2 mg/kg, i.v., Sigma) dissolved in saline (Ahmed et al., 2010; Aubert et al., 2005; Bezard et al. 2001, 2003; Porras et al., 2012; Shen et al., 2015; Urs et al., 2015). Daily (9 a.m.) assessment of parkinsonism was performed in home cages for 30 min by two blinded observers using a validated rating scale (Bezard et al., 2001; Imbert et al., 2000) assessing tremor, general level of activity, body posture (flexion of spine), vocalization, freezing and frequency of arm movements and rigidity (for each upper limb). Following a 3-month stabilization of the MPTP-induced syndrome, 4 animals were used together with 4 control animals for the further functional investigations. We waited at least 6 days between tests. A general observation of each animal in its home cage was performed before each blood sampling. Any observable side-effect was documented and timed.

2.2. Bristol stool scale

The Bristol scale was used to evaluate the transit diary and score the stool once a day for 4–8 weeks. Bristol scale rate stools depending on its shape and constituency from 1 (hard, solid stool) to 7 (liquid faeces). 3, 4 and 5 are considered as normal faeces, 1 and 2 as constipation and 6 and 7 as diarrhea (Blake et al. 2016). Days of acetaminophen, FITC-dextran and insulin administration were excluded from analysis. Stools were graded from 1 to 7 depending on the shape and consistency.

2.3. Gastric emptying rate: acetaminophen absorption test

Animals were fasted overnight for 16 h before acetaminophen administration and 6 h after dosing. To avoid pharmacological bias, animals were OFF-levodopa when tested. Acetaminophen (60 mg/kg PO) (Ikegami et al., 2003) was administered orally by gavage at a volume of 5 mL/kg. Blood samples (approximately 1 mL) were taken immediately before acetaminophen administration and 15, 30, 45, 60, 90, 120, 240 and 360 min afterwards. Samples were centrifuged within 30 min after collection at 3000 g for 10 min at 4 °C and plasma samples were kept frozen at -20 °C until analysis. An 30 μL aliquot was added to 150 μL IS (Osalmid or Propranolol, 40 ng/mL) in Acetonitrile (ACN). The mixture was vortexed for 5 min and centrifuged at 5800 rpm for 10 min. A 3 μL sample was injected into the liquid chromatography-tandem mass spectrometry system (API5500, Qtrap). Each sample concentration was calculated by comparison to the calibration curve (10.00–30000 ng/mL). Concentration data under 10.0 ng/mL for acetaminophen was replaced with "below quantifiable limit (BQL)" and excluded from graphing and estimation of PK parameters.

2.4. Measurement of intestinal permeability: FITC-dextran test

Animals were fasted overnight for 16 h before acetaminophen administration and 6 h after dosing. Animals were OFF-levodopa when tested. Animals were dosed orally by gavage with 400 mg/kg FITC-dextran solution (4 kDa) at a volume of 10 ml/kg (Feng et al., 2019; Cladis et al., 2020; Joly Condette et al., 2014). Blood samples were collected before and at 0.5, 1, 2, 4h post-administration into EDTA-coated tubes and kept on ice. Within 10 min of collection, whole blood samples were centrifuged at 3000 g for 10 min at 4 °C and the resulting plasma samples were aliquoted into polypropylene tubes containing at least 100 μL of plasma for FITC concentration determination. Samples were kept frozen at –20 °C until analysis. FITC-dextran levels in plasma were quantified using HPLC with fluorescence detection (HPLC-FLD). A 100 μL aliquot was added to 20 μL trichloroacetic acid (TCA). The mixture was vortexed for 5 min and centrifuged at 5800 rpm for 10 min. 80 μL supernatant was added to 20 μL NaOH. 5 μL of the mixture was injected into the liquid chromatography fluorescence detector (LC-FLD). Each sample concentration was calculated by comparison to the calibration curve (50.00–10000 ng/mL).

2.5. Intravenous glucose tolerance test (IVGTT)

Animals were fasted overnight for 16 h before the intravenous glucose tolerance test (IVGTT). Animals were OFF-levodopa when tested. On the day of administration, the monkeys were anesthetized with ketamine (10 mg/kg, IM) one hour ahead of the IVGTT procedure and placed on a flat table in preparation for IVGTT. Blood samples were taken3 min prior to the IVGTT for baseline glucose and insulin measurements. The injection area was disinfected and cleansed with iodine and 75% alcohol solution. A 50% glucose solution was administered at 0.5 g/kg of glucose into a peripheral vein for 3 min. Blood samples were collected at the following time points after the end of the glucose IV bolus injection: 1, 5, 10, 20, 40 and 60 min.

At each specified time point, 1 mL of blood was collected from the vein directly into a prepared tube containing 500 KIU (Kallikrein Inhibitor Unit) aprotinin. The vacutainer was then gently inverted 3–5 times and placed on wet ice. Plasma was separated by centrifugation (i.e. 3000 g for 10 min at 4 °C) within 30 min of collection. The concentrations of glucose were determined using Automated MINDRAY BS-380. Concentrations of glucose below 0.6 mmol/l were considered BLQ. The concentrations of insulin and C-peptide were determined using Monkey Insulin ELISA Kit (Colorimetric) and Monkey C-Peptide ELISA Kit (Colorimetric), respectively, from NOVUS BIOLOGICALS, according to manufacturer's procedures. Plates were read at 450 nm with SoftMax Pro GxP 5.4.4. Concentrations below 30 μIU/mL for insulin and 0.21 ng/mL for c-peptide were considered BQL.

2.6. Statistics

Statistics were performed using GraphPad Prism 8 Software. Data are represented in text and figures as medians (95% Confidence Interval). Mann-Witney tests were used to compare the data between the two groups. Chi-square test was performed to compare stool consistency distribution between groups.

3. Results

3.1. MPTP-induced parkinsonism

MPTP macaques were all parkinsonian and positively responded to L-DOPA as previously published (Ahmed et al., 2010; Aubert et al., 2005; Bezard et al. 2001, 2003; Porras et al., 2012; Shen et al., 2015; Urs et al., 2015), with a near complete reversal of parkinsonism peaking at 60 min post-intake progressively resurging until being turned OFF again around 240 min post-intake.

3.2. Bristol stool scale

During the study, most stools were rated between 1 and 4 in the Bristol scale. Distribution of stool ratings (Figure 1A, B) is significantly different between groups ($p = 0.0003$). MPTP animals presented less stools rated as 1–2 (hard lump) than controls (35% (27–51%) vs 64% (62–66%), $p = 0.03$). Number of stools per day was slightly increased in MPTP versus controls (1.64 (1.57–1.70) vs 1.43 (1.27–1.48), $p = 0.03$). Altogether, MPTP monkeys did not exhibited signs of slower transit time but rather of an acceleration of the transit.

3.3. Acetaminophen absorption test

Acetaminophen is not absorbed from the stomach but from the small intestine. The kinetics in blood following single oral dosing is considered a measure of gastric emptying rate (Clements et al., 1978; Hatanaka et al., 1994; Heading et al., 1973). In MPTP monkeys, acetaminophen reached a maximum plasma concentration of 34,750 ng/mL (95%CI from 30,200 to 59,400 ng/mL) after a median of 1.5 h (95%CI from 0.25 to 2 h) post-administration (Figure 1A). In control monkeys, a maximum concentration of 38,250 ng/mL (95%CI from 21,700 to 45,000 ng/mL) was obtained 0.625 h (95%CI from 0.5 to 2 h) after administration (Figure 2A). Time to peak concentration was not statistically different between groups ($p = 0.83$, Figure 2B). This test did not reveal any statistic gastric emptying rate difference between control and MPTP monkeys. However, inter-animal variability was high and 3 out of 4 MPTP animals had a delayed peak (1.5 h or later) compared to 3 out of 4 control animals (less than 1 h).

3.4. FITC-dextran test

FITC-dextran is a large macromolecule which does not readily pass through a healthy gut barrier. Its appearance in plasma after oral administration is suggestive of a compromised intestinal barrier and paracellular permeability (Woting and Blaut 2018; Li et al., 2018; Volynets et al., 2016). The FITC-dextran fluorescence level was evaluated from 0 to 4 h post-absorption with no statistical difference between MPTP monkey and controls (4 h post-administration, Mann-Witney $p = 0.06$, Figure 2C). There was a trend towards decreased area under the curve (AUC) in MPTP animals (AUC = 1,321 ng/mL, 95%CI from 1,028 to 1,971 ng/mL) versus control animals (AUC = 3,055 ng/mL, 95%CI from 1,170 to 8,450 ng/mL, $p = 0.06$, Figure 2D). Altogether, no altered permeability was detected in parkinsonian monkeys.

3.5. IVGTT

After an IV injection of glucose, no difference in plasma glucose clearance (Figure 3A) or AUC (Figure 3B) was observed between MPTP and control monkeys. C-peptide was not detectable and insulin was below the limit of detection for most of blood samples in MPTP monkeys but detectable in controls (data not shown). Statistical analysis could therefore not be performed. From a qualitative point of view, insulin levels might be lower in MPTP monkeys compared to controls. Altogether, these observations suggest that glycaemic response to an IV injection of glucose did not differ between groups.

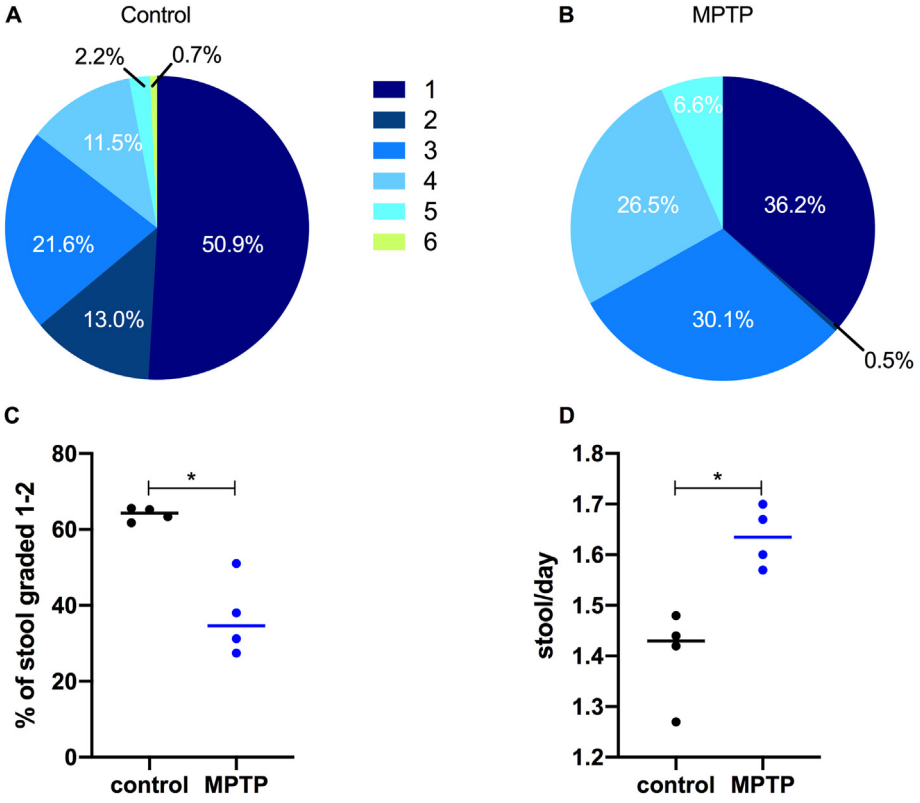

Figure 1. Bristol stool scale. A. Stool classification in control (A) and MPTP (B) animals. C. Percentage of stools graded as 1 or 2 in Bristol scale ($p = 0.03$). D. Number of stools per animal and per day ($p = 0.03$).

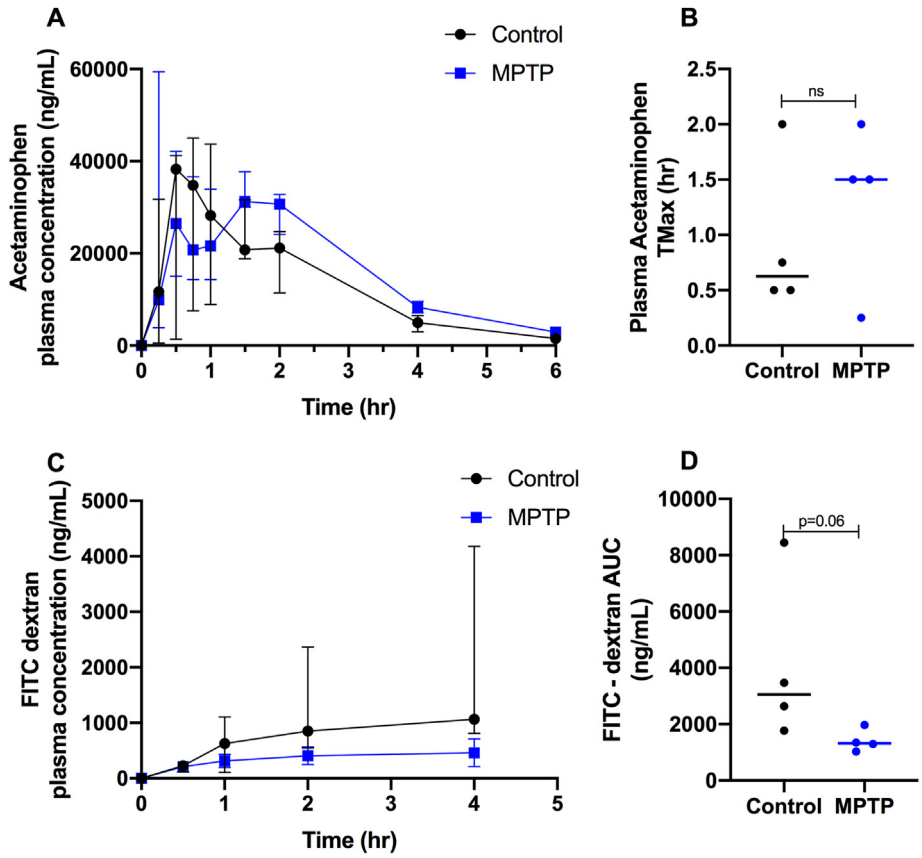

Figure 2. Acetaminophen and FITC-dextran absorption tests. A. Kinetics of plasma acetaminophen in MPTP and control primates. B. Tmax (time to maximal concentration) of acetaminophen ($p = 0.83$). C. Kinetics of plasma FITC-Dextran in control and MPTP monkeys. D. Area under the curve (AUC) of FITC-Dextran ($p = 0.06$).

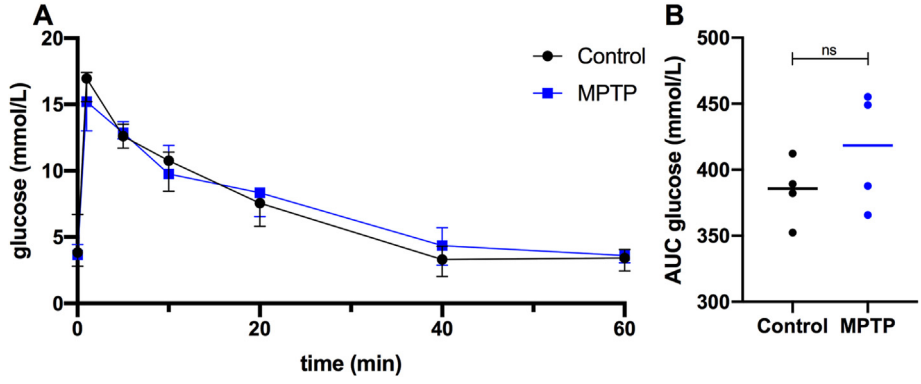

Figure 3. Intra-veinous glucose tolerance test. A. Kinetics of plasma glucose after IV administration of 0.5 mg/kg glucose ($p > 0.05$, ns). B. glucose AUC during IVGTT ($p = 0.49$).

4. Discussion

In this study, we did not find any significant dysfunction in gastric emptying rate or intestinal permeability in MPTP monkeys. Indeed, neither the acetaminophen nor FITC-dextran absorption test showed a difference between control and MPTP animals. There was no difference between control and MPTP groups in insulin sensitivity, as measured using the IVGTT. Moreover, MPTP monkeys did not display the constipation that occurs in PD patients.

PD is a complex disease, with causative factors still unknown (Kalia and Lang 2015). Genetic susceptibility and environmental injury may play a role and modeling such a disease in animals is challenging. Animal models are based on either genetic mutations responsible for rare familial forms of PD or on injection of toxins to reproduce dopaminergic cell death (McDowell and Chesselet 2012). Within these toxicity models of PD, toxin administration is either intra-cerebral (e.g. 6-hydroxydopamine injections in substantia nigra or striatum in rodents) or systemic (e.g. MPTP in primates). These systemic models might be more valuable to model PD, as the disease is not limited to the CNS but is also found systemically, like in the gut (Lebouvier et al., 2008).

It is noteworthy that the MPTP-treated mouse was previously shown to display a transient acceleration of colonic transit (Anderson et al., 2007). These transit changes might be more prolonged in MPTP-treated monkeys. GI function alteration in PD is complex and might involve all the GI segments. In particular, constipation, delayed gastric emptying and increased intestinal permeability have been described (Fasano et al.,

2015; Pfeiffer 2018; Forsyth et al., 2011; van IJzendoorn and Derkinderen 2019). We here show that, in a stabilized parkinsonian syndrome, MPTP-treated monkeys do not model GI symptoms, although lesions of the enteric nervous system have previously been reported. Indeed, MPTP-treated monkeys showed a reduction in the number of tyrosine hydroxylase positive (TH+) neurons, i.e. likely dopaminergic neurons, in the colonic enteric nervous system, mainly in the myenteric plexus (Chaumette et al., 2009). Ex-vivo analyses of contractility of the longitudinal smooth muscle of the ileum in MPTP-treated marmoset did reveal changes compared to control animals that might be due to nitrergic transmission alteration (Coletto et al., 2019). It, thus, appears that these neuropathological enteric nervous system lesions and physiological and biochemical alterations are not sufficient to induce parkinsonian GI symptoms in monkeys. Interestingly, autonomic nervous system, cardiac in particular, was shown to be altered in MPTP-treated monkey (Carmona-Abellan et al., 2019).

One can therefore hypothesize that either the nature of the enteric nervous system lesion in MPTP monkeys is different to that in PD patients or that the extent of the enteric nervous system lesion in the MPTP monkey is not sufficient to reach symptomatic threshold. It is also worth taking into consideration the fluctuating nature of these symptoms in humans, suggesting that a longitudinal study in the MPTP monkey might be more appropriate than single-point measures as performed in the present study although the Bristol scale was the only endpoint capable to distinguish between control and MPTP monkeys. However, according to Saad and colleagues, stool form correlated weakly with transit time and only in constipated humans, whereas stool frequency did not reflect properly transit time (Saad et al., 2010).

One should keep in mind that this study was exploratory, based on a small effective of animals. Increasing the size of the study would have helped to see any statistically significant difference.

5. Conclusion

Although the MPTP-treated monkey model of PD is considered the gold standard to test potential therapeutics aiming at alleviating several motor and non-motor symptoms, the current study did not demonstrate PD-related alterations in GI function or insulin sensitivity in this model, highlighting that a fantastic phenocopy of a syndrome might remain imperfect facing the complexity of a human condition.

Declarations

Author contribution statement

Anna Delamarre, Qin Li: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Cliona MacSweeney, Rie Suzuki, Alastair JH Brown: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Elsa Y Pioli, Erwan Bezard: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Funding statement

Erwan Bezard was supported by Motac Neuroscience Ltd. Cliona MacSweeney was supported by Sosei Heptares Ltd.

Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

Abbott, R.D., Petrovitch, H., White, L.R., Masaki, K.H., Tanner, C.M., Curb, J.D., Grandinetti, A., Blanchette, P.L., Popper, J.S., Ross, G.W., 2001. Frequency of bowel movements and the future risk of Parkinson's disease. Neurology 57, 456–462.

Ahmed, M.R., Berthet, A., Bychkov, E., Porras, G., Li, Q., Bioulac, B.H., Carl, Y.T., Bloch, B., Kook, S., Aubert, I., Dovero, S., Doudnikoff, E., Gurevich, V.V., Gurevich, E.V., Bezard, E., 2010. Lentiviral overexpression of GRK6 alleviates L-dopa-induced dyskinesia in experimental Parkinson's disease. Sci. Transl. Med. 2, 28ra28.

Anderson, G., Noorian, A.R., Taylor, G., Anitha, M., Bernhard, D., Srinivasan, S., Greene, J.G., 2007. Loss of enteric dopaminergic neurons and associated changes in colon motility in an MPTP mouse model of Parkinson's disease. Exp. Neurol. 207, 4–12.

Aubert, I., Guigoni, C., Hakansson, K., Li, Q., Dovero, S., Barthe, N., Bioulac, B.H., Gross, C.E., Fisone, G., Bloch, B., Bezard, E., 2005. Increased D1 dopamine receptor signaling in levodopa-induced dyskinesia. Ann. Neurol. 57, 17–26.

Bezard, E., Ferry, S., Mach, U., Stark, H., Leriche, L., Boraud, T., Gross, C., Sokoloff, P., 2003. Attenuation of levodopa-induced dyskinesia by normalizing dopamine D3 receptor function. Nat. Med. 9, 762–767.

Bezard, Erwan, Dovero, Sandra, Prunier, Caroline, Ravenscroft, Paula, Chalon, Sylvie, Guilloteau, Denis, Crossman, Alan R., Bernard, Bioulac, Brotchie, Jonathan M., Gross, Christian E., 2001. Relationship between the appearance of symptoms and the level of nigrostriatal degeneration in a progressive 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned macaque model of Parkinson's disease'. J. Neurosci. 21, 6853–6861.

Blake, M.R., Raker, J.M., Whelan, K., 2016. Validity and reliability of the Bristol Stool Form Scale in healthy adults and patients with diarrhoea-predominant irritable bowel syndrome. Aliment. Pharmacol. Ther. 44, 693–703.

Carmona-Abellan, M., Martinez-Valbuena, I., DiCaudo, C., Marcilla, I., Luquin, M.R., 2019. Cardiac sympathetic innervation in the MPTP non-human primate model of Parkinson disease. Clin. Auton. Res. 29, 415–425.

Chaumette, T., Lebouvier, T., Aubert, P., Lardeux, B., Qin, C., Li, Q., Accary, D., Bezard, E., Bruley des Varannes, S., Derkinderen, P., Neunlist, M., 2009. Neurochemical plasticity in the enteric nervous system of a primate animal model of experimental Parkinsonism. Neuro Gastroenterol. Motil. 21, 215–222.

Cladis, D.P., Li, S., Reddivari, L., Cox, A., Ferruzzi, M.G., Weaver, C.M., 2020. A 90 day oral toxicity study of blueberry polyphenols in ovariectomized sprague-dawley rats. Food Chem. Toxicol. 139, 111254.

Clements, J.A., Heading, R.C., Nimmo, W.S., Prescott, L.F., 1978. Kinetics of acetaminophen absorption and gastric emptying in man. Clin. Pharmacol. Ther. 24, 420–431.

Coletto, E., Dolan, J.S., Pritchard, S., Gant, A., Hikima, A., Jackson, M.J., Benham, C.D., Chaudhuri, K.R., Rose, S., Jenner, P., Iravani, M.M., 2019. Contractile dysfunction and nitrergic dysregulation in small intestine of a primate model of Parkinson's disease. NPJ Parkinsons Dis. 5, 10.

Davies, K.N., King, D., Billington, D., Barrett, J.A., 1996. Intestinal permeability and orocaecal transit time in elderly patients with Parkinson's disease. Postgrad. Med. J. 72, 164–167.

Engeln, M., De Deurwaerdere, P., Li, Q., Bezard, E., Fernagut, P.O., 2015. Widespread monoaminergic dysregulation of both motor and non-motor circuits in parkinsonism and dyskinesia. Cerebr. Cortex 25, 2783–2792.

Fasano, Alfonso, Visanji, Naomi P., Liu, Louis W.C., Lang, Antony E., Pfeiffer, Ronald F., 2015. Gastrointestinal dysfunction in Parkinson's disease. Lancet Neurol. 14, 625–639.

Feng, X.Y., Yang, J., Zhang, X., Zhu, J., 2019. Gastrointestinal non-motor dysfunction in Parkinson's disease model rats with 6-hydroxydopamine. Physiol. Res. 68, 295–303.

Forsyth, C.B., Shannon, K.M., Kordower, J.H., Voigt, R.M., Shaikh, M., Jaglin, J.A., Estes, J.D., Dodiya, H.B., Keshavarzian, A., 2011. Increased intestinal permeability correlates with sigmoid mucosa alpha-synuclein staining and endotoxin exposure markers in early Parkinson's disease. PloS One 6, e28032.

Greene, J.G., 2011. Animal models of gastrointestinal problems in Parkinson's disease. J. Parkinsons Dis. 1, 137–149.

Hatanaka, Satoshi, Kondoh, Masaki, Kawarabayashi, Keiko, Furuhama, Kazuhisa, 1994. The measurement of gastric emptying in conscious rats by monitoring serial changes in serum acetaminophen level. J. Pharmacol. Toxicol. Methods 31, 161–165.

Heading, R.C., Nimmo, J., Prescott, L.F., Tothill, P., 1973. The dependence of paracetamol absorption on the rate of gastric emptying. Br. J. Pharmacol. 47, 415–421.

Ikegami, K., Tagawa, K., Narisawa, S., Osawa, T., 2003. Suitability of the cynomolgus monkey as an animal model for drug absorption studies of oral dosage forms from the viewpoint of gastrointestinal physiology. Biol. Pharm. Bull. 26, 1442–1447.

Imbert, Christelle, Bezard, Erwan, Guitraud, Stéphane, Boraud, Thomas, Christian, E., Gross, 2000. Comparison of eight clinical rating scales used for the assessment of MPTP-induced parkinsonism in the Macaque monkey. J. Neurosci. Methods 96, 71–76.

Joly Condette, C., Khorsi-Cauet, H., Morliere, P., Zabijak, L., Reygner, J., Bach, V., Gay-Queheillard, J., 2014. Increased gut permeability and bacterial translocation after chronic chlorpyrifos exposure in rats. PloS One 9, e102217.

Kalia, Lorraine V., Lang, Anthony E., 2015. Parkinson's disease. Lancet 386, 896–912.

Lebouvier, T., Chaumette, T., Damier, P., Coron, E., Touchefeu, Y., Vrignaud, S., Naveilhan, P., Galmiche, J.P., Bruley des Varannes, S., Derkinderen, P., Neunlist, M.,

2008. Pathological lesions in colonic biopsies during Parkinson's disease. Gut 57, 1741–1743.
Li, B.R., Wu, J., Li, H.S., Jiang, Z.H., Zhou, X.M., Xu, C.H., Ding, N., Zha, J.M., He, W.Q., 2018. In vitro and in vivo approaches to determine intestinal epithelial cell permeability. J. Vis. Exp.
McDowell, K., Chesselet, M.F., 2012. 'Animal models of the non-motor features of Parkinson's disease. Neurobiol. Dis. 46, 597–606.
Pfeiffer, R.F., 2018. 'Gastrointestinal dysfunction in Parkinson's disease'. Curr. Treat. Options Neurol. 20, 54.
Porras, G., Berthet, A., Dehay, B., Li, Q., Ladepeche, L., Normand, E., Dovero, S., Martinez, A., Doudnikoff, E., Martin-Negrier, M.L., Chuan, Q., Bloch, B., Choquet, D., Boue-Grabot, E., Groc, L., Bezard, E., 2012. PSD-95 expression controls L-DOPA dyskinesia through dopamine D1 receptor trafficking. J. Clin. Invest. 122, 3977–3989.
Saad, R.J., Rao, S.S., Koch, K.L., Kuo, B., Parkman, H.P., McCallum, R.W., Sitrin, M.D., Wilding, G.E., Semler, J.R., Chey, W.D., 2010. Do stool form and frequency correlate with whole-gut and colonic transit? Results from a multicenter study in constipated individuals and healthy controls. Am. J. Gastroenterol. 105, 403–411.
Shen, W., Plotkin, J.L., Francardo, V., Ko, W.K., Xie, Z., Li, Q., Fieblinger, T., Wess, J., Neubig, R.R., Lindsley, C.W., Conn, P.J., Greengard, P., Bezard, E., Cenci, M.A., Surmeier, D.J., 2015. M4 muscarinic receptor signaling ameliorates striatal plasticity deficits in models of L-DOPA-induced dyskinesia. Neuron 88, 762–773.
Urs, N.M., Bido, S., Peterson, S.M., Daigle, T.L., Bass, C.E., Gainetdinov, R.R., Bezard, E., Caron, M.G., 2015. Targeting beta-arrestin2 in the treatment of L-DOPA-induced dyskinesia in Parkinson's disease. Proc. Natl. Acad. Sci. U. S. A. 112, E2517–E2526.
van IJzendoorn, S.C.D., Derkinderen, P., 2019. The intestinal barrier in Parkinson's disease: current state of knowledge. J. Parkinsons Dis. 9, S323–S329.
Volynets, V., Reichold, A., Bardos, G., Rings, A., Bleich, A., Bischoff, S.C., 2016. Assessment of the intestinal barrier with five different permeability tests in healthy C57BL/6J and BALB/cJ mice. Dig. Dis. Sci. 61, 737–746.
Woting, A., Blaut, M., 2018. Small intestinal permeability and gut-transit time determined with low and high molecular weight Fluorescein isothiocyanate-dextrans in C3H mice. Nutrients 10.