Neurotoxicology 91 (2022) 166–176

Contents lists available at ScienceDirect

Neurotoxicology

journal homepage: www.elsevier.com/locate/neuro

Spontaneous partial recovery of striatal dopaminergic uptake despite nigral cell loss in asymptomatic MPTP-lesioned female minipigs

Thea P. Lillethorup [a,b], Ove Noer [a], Aage Kristian Olsen Alstrup [a], Caroline C. Real [a,b], Kathrine Stokholm [a], Majken Borup Thomsen [a,b], Hamed Zaer [c], Dariusz Orlowski [c], Trine Werenberg Mikkelsen [c], Andreas N. Glud [c], Erik Holm Toustrup Nielsen [a], Anna C. Schacht [a], Michael Winterdahl [a,b], David J. Brooks [a,d], Jens Christian H. Sørensen [c], Anne M. Landau [a,b,*]

[a] Department of Nuclear Medicine & PET-Center, Department of Clinical Medicine, Aarhus University Hospital, 8200 Aarhus N, Denmark
[b] Translational Neuropsychiatry Unit, Department of Clinical Medicine, Aarhus University, 8000 Aarhus C, Denmark
[c] Department of Neurosurgery and Center for Experimental Neuroscience (CENSE), Aarhus University Hospital, 8200 Aarhus N, Denmark
[d] University of Newcastle upon Tyne, UK

ARTICLE INFO

Edited by William Slikker Jr.

Keywords:
Dopamine
MPTP
Parkinson's disease
Positron emission tomography
Vesicular monoamine transporter 2
Minipig

ABSTRACT

The Göttingen minipig is a large animal with a gyrencephalic brain that expresses –complex behavior, making it an attractive model for Parkinson's disease research. Here, we investigate the temporal evolution of presynaptic dopaminergic function for 14 months after injections of 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) into the minipig using a multi-tracer longitudinal positron emission tomography (PET) design. We injected seven sedated minipigs with 1–2 mg/kg of MPTP, and two with saline, three times a week over four weeks. We monitored behavioral deficits using a validated motor scale and walking mat. Brains were imaged with (+)-α-[^{11}C]-dihydrotetrabenazine ([^{11}C]-DTBZ) and [^{18}F]-dihydroxyphenylalanine ([^{18}F]-FDOPA) PET at baseline and 1, 3, 10 and 14 months after MPTP injection, and immunohistochemistry was used to assess nigral cell loss. The minipigs showed mild bradykinesia and impaired coordination at early timepoints after MPTP. PET revealed decreases of striatal [^{11}C]-DTBZ and [^{18}F]-FDOPA uptake post-MPTP with partial spontaneous recovery of [^{18}F]-FDOPA after 10 months. Postmortem analysis estimated an MPTP-induced nigral loss of 57% tyrosine hydroxylase+ and 43% Nissl-stained cells. Normal motor function despite substantial damage to the dopaminergic system is consistent with prodromal Parkinson's disease, and offers an opportunity for testing disease-modifying therapies. However, partial spontaneous recovery of dopamine terminal function must be taken into account in future studies.

1. Introduction

Parkinson's disease (PD) is a chronic neurodegenerative disease characterized by degeneration of nigro-striatal dopaminergic neurons and associated with motor symptoms including bradykinesia, rigidity and tremor. The 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of PD in non-human primates is a well-established animal lesion model of PD (Konnova and Swanberg, 2018). MPTP is metabolized to a toxic by-product, MPP+ , which enters dopaminergic neurons and induces neuronal degeneration by blocking complex 1 activity of the mitochondrial electron transport chain (Ramsay et al., 1986). The model presents with parkinsonian motor symptoms, shows loss of nigral dopamine neurons and striatal levels of dopamine, and responds to levodopa therapy, making it an attractive model system with which to evaluate the efficacy of neuroprotective agents (Burns et al., 1983; Doudet et al., 1985; Landau et al., 2012; Langston et al., 1984). MPTP also induces dopamine deficiency in mice (Landau et al., 2005; Lau and Fung, 1986; Lau et al., 1991) and a number of other small species, including the goldfish (Weinreb and Youdim, 2007). Non-human primates are often the preferred species for the MPTP model, but low availability, ethical, and cost issues limit their use (Harding, 2017). The Göttingen minipig is a strain specifically bred for research as their

* Correspondence to: Translational Neuropsychiatry Unit, Aarhus University, Universitetsbyen 13, 2b, 8000 Aarhus C, Denmark.
E-mail address: alandau@clin.au.dk (A.M. Landau).

https://doi.org/10.1016/j.neuro.2022.05.006
Received 14 February 2022; Received in revised form 6 April 2022; Accepted 9 May 2022
Available online 13 May 2022

limited growth permits long-term longitudinal follow-up with non-invasive neuroimaging while their brain size allows identification of the sub-regional striatal distribution of pre- and post-synaptic dopaminergic tracers (Alstrup et al., 2010; Landau et al., 2018; Lillethorup et al., 2018b). Our team has previously developed and validated MPTP-lesion pig models and studied the efficacy of stem cell transplantation and deep brain stimulation of the subthalamic nucleus (Dall et al., 2002; Danielsen et al., 2000; Mikkelsen et al., 1999; Nielsen et al., 2016).

In the current report, we studied the longitudinal effects of MPTP administration to minipigs on behavior and uptake of presynaptic markers of the dopamine system known to be affected in human PD, using serial positron emission tomography (PET) over a 14-month period. Despite large doses of MPTP, the minipigs became only mildly symptomatic at early stages after MPTP injections. Exogenous dopa decarboxylation in dopaminergic terminals was serially studied with fluoro-3,4-dihydroxyphenylalnine ([^{18}F]-FDOPA) PET (Doudet et al., 1998) while vesicular monoamine transporter 2 (VMAT2) availability was measured with (+)-α-[^{11}C]-dihydrotetrabenazine ([^{11}C]-DTBZ) PET (Doudet et al., 2006). The longitudinal PET data are discussed in light of the mild behavioral changes and neuropathology of the minipigs after MPTP and compared to reported findings in both minipig and non-human primates.

2. Materials and methods

2.1. Animals

We studied nine 1-year old female Göttingen minipigs (Ellegaard Minipigs ApS, Dalmose, Denmark) weighing 25 kg at the beginning of the experiment. This study was approved and regulated by The Danish Council for Animal Experiments (License: 2016−15−0201−00878) and all experiments were carried out in accordance with Danish law and regulations for the Protection of Animals used for Scientific Purposes (Animal inspectorate), the ARRIVE guidelines and the 2010/63/EU directive for animal experiments. An animal technician and veterinarian monitored animals daily and weekly, respectively. The pigs were acclimatized for several weeks prior to the experiment. We fed minipigs a restricted pellet diet (SDS Diet, Witham, UK) and fasted them overnight, with free access to tap water, prior to the day of the experiment. We group-housed pigs in a 4.6 m^2 enclosure with fence-line visual contact to one another under environmental conditions of 20 °C and 50–55% relative humidity, with air changed 8 times every hour and 12-hour light/dark cycles. We sedated minipigs during subcutaneous MPTP- or saline-injections and anesthetized them for the scanning procedures. We followed humane endpoints of weight loss of over 10% body weight in combination with other observations suggestive of poor well-being, bleeding complications or signs of hypoxia under anaesthesia. All minipigs were clinically healthy prior to the study, and they were randomized to either MPTP or saline treatment groups.

2.2. Study overview

Minipigs were imaged at baseline with [^{18}F]-FDOPA and [^{11}C]-DTBZ PET and then injected 3 times per week with 1–2 mg/kg MPTP over a 4-week period (Fig. 1). Due to the radiochemistry schedule, it was not possible to conduct PET with both radioligands in each animal at each timepoint and one [^{18}F]-FDOPA scan was excluded due to an acquisition error. This led to a varying number of scans across the animals. The MPTP group PET data was obtained with group sizes of at least 5 minipigs (5–6 for baseline and 6–7 for post MPTP scans). Neurological scoring and gait were assessed throughout the study. Immunohistochemistry with tyrosine hydroxylase (TH) and Nissl-staining were performed to assess nigral cell death.

The MPTP-injected minipigs were part of a study of the effects of human engineered stem cells as therapeutic agents in the MPTP pig model of PD. Human embryonic progenitor derived DA neurons were bilaterally injected in three of the MPTP intoxicated minipigs (Nolbrant et al., 2017). Due to lack of behavioral symptoms, spontaneous partial recovery of striatal dopaminergic neurotransmission, and insufficient immunosuppression (>2 μg/l tacrolimus blood level), there were no differences between the minipigs that received stem cells and sham treatment in any of the studied parameters, and we found no histological evidence of stem cell survival. We, therefore, pooled the data for the purpose of this study to investigate the long-term effects of MPTP on [^{18}F]-FDOPA and [^{11}C]-DTBZ binding, and potential recovery of PET measures in near asymptomatic MPTP-injected minipigs. For transparency, the minipigs receiving stem cells and sham surgery are marked with different icons in each graph.

2.3. MPTP administration

Three times a week over a 4-week period, we sedated the minipigs with approximately 0.8 mg/kg of midazolam (Hameln) and 5 mg/kg of S-ketamine (Pfizer) or 1.2–2.8 mg/kg midazolam alone. We injected 1 mg/kg of MPTP (BioNordika Denmark A/S, Herlev) for the first 2 weeks and then 2 mg/kg for the last 2 weeks resulting in a cumulative dose of 18 mg/kg subcutaneously into the hind leg groin (SC) designed to induce a stable syndrome of parkinsonism. Control pigs had an equivalent volume of saline injected.

2.4. Neurological score and behavioral impairment

During the intoxication period and approximately every week after MPTP or saline injections, we scored the neurological status of the minipigs. This was done over a 5-minute period for each minipig on 60 separate days (over 478 days) by an observer with veterinary experience in the evaluation of healthy and sick minipigs, and who was blind to the treatment groups. We used previously published protocols (Dall et al., 2002; Danielsen et al., 2000; Mikkelsen et al., 1999) with a few modifications (Table 1). The experienced observer also noted changes to fecal output and whether food was consumed, but these did not factor into the score.

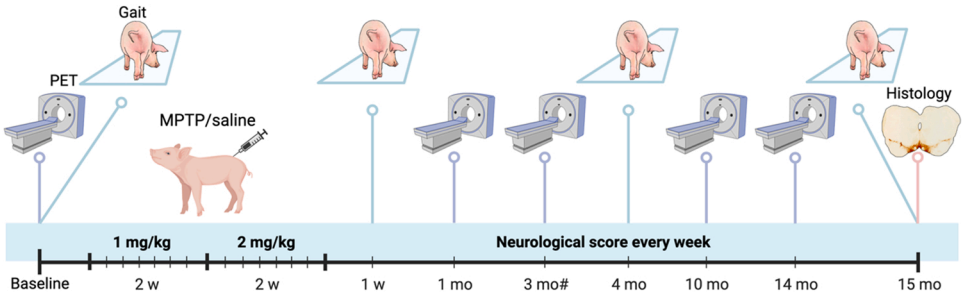


Fig. 1. Overview of the study timeline. The minipigs were behaviorally tested on the gait mat and scanned with [^{18}F]-FDOPA and [^{11}C]-DTBZ PET at baseline. Then, they received either MPTP (n = 7) or saline (n = 2) 3 times/week over 4 weeks. They underwent an approximate weekly neurological score throughout the study. Over the next 15 months, the minipigs were followed with PET and gait analysis and the brains were removed for immunohistochemical staning after 15 months. #Only MPTP-intoxicated minipigs were scanned at 3 months. Created with BioRender.com.

Table 1
Neurological status rating scale.

Behavior	Observation	Score
Motility	Normal	0
	Slightly compromised	1
	Moderately compromised	2
	Severely compromised	3
	Lean against wall for support	4
	Freezing of muscles	5
Position abnormality/ Coordination	Normal	0
	Abnormal leg positioning (i.e. crossing of hindlimbs)	1
	Lowered head	2
	Clear coordination issues	3
Muscle rigidity	Normal	0
	Slight rigidity	1
	Severe rigidity	2
Chewing function	Normal	0
	Slightly compromised chewing function	1
	Rigid jaws and reduced chewing	2
Vocalisations	Normal	0
	Altered voice/sounds	1
	Dramatically altered voice/sounds	2
Total score	Normal	0
	Mild	1–4
	Moderate	5–10
	Severe	11–14

We also assessed walking speed and total pressure index (TPI) of the gait using a GAIT4Dog walking mat prior to any treatments and at 1 week, 4 months and 15 months after the last MPTP or saline treatment. Minipigs walked at their preferred velocity on the pressure sensing walkway system. Three consecutive gait cycles (12 steps) or more accounted for one quality reading and the final measures were an average of at least 3 quality readings per animal.

2.5. PET imaging

As previously described (Landau et al., 2018), minipigs were anesthetized with a mixture of 1.25 mg/kg midazolam (Hameln) and 6.25 mg/kg S-ketamine (Pfizer) IM, intubated for mechanical ventilation and prepared for PET imaging. After intubation, the anaesthesia was maintained with 2.0–2.1% isoflurane. The pigs were mechanically ventilated with approximately 8 mL/kg of a 1: 2.2 oxygen-medical air mixture. We monitored physiological parameters which all remained at normal porcine values (Alstrup, 2010). We placed minipigs supine in a human PET/CT scanner (Siemens Biograph 64 Truepoint PET) and immobilized their head and body. We positioned the animal within the field of view of the PET camera and performed a low-dose CT scan prior to each PET recording for anatomical definition and attenuation correction of PET emission data.

We intravenously administered an average of 369 ± 26 MBq (range 315–415 MBq) (+)-α-[^{11}C] labeled DTBZ (saline with 10% ethanol) via an ear vein catheter in 10 mL saline, during the initial 60 s of a 90-minute scan. After the DTBZ scan, we intravenously administered carbidopa (50 mg/10 mL, 42 mM NaH_2PO_4, pH 3) in each animal to reduce their peripheral DOPA decarboxylase activity and then after 30 min scanned for 90 min with an average of 199 ± 22 MBq (range 107–222 MBq) [^{18}F] labeled FDOPA (70 mM NaH_2PO_4, pH 4.5). [^{11}C]-DTBZ data was collected first due to the shorter 20-minute half-life of [^{11}C]. We reconstructed PET data using TrueX 3D OSEM (3 iterations), a 256×256×109 matrix, and a 2-mm Gauss filter, using a time-frame structure of 5 × 60, 3 × 300, 4 × 600, 2 × 900 s (total 14 frames, 90 min).

2.6. Quantitative PET analyses

PET studies were analysed using routine existing procedures for pigs developed in Aarhus (Landau et al., 2019; Lillethorup et al., 2018a, 2018b) by an experimenter blinded to the treatment groups. We performed all preprocessing steps using PMOD™ 3.7 (PMOD Technologies Ltd, Zurich, Switzerland), a commercially available software package for biomedical image quantification. To define the stereotaxic transformation parameters from time-averaged PET images of [^{11}C]-DTBZ, we used a ligand-specific template. We applied the generated transformation matrices and warping fields onto the corresponding dynamic PET time series. We generated parametric BP_{ND} images of [^{11}C]-DTBZ binding using the Simplified Reference Tissue Model 2 (SRTM2). [^{18}F]-FDOPA data was analysed using Patlak reference plots with t* set at 30 min as the time for the tracer to equilibrate between the plasma and brain free compartments. Based on the atlas by Watanabe et al. (2001), we created custom-made masks of the cerebellum to obtain the cerebellar tissue radioactivity as a region of non-displaceable binding and extracted time activity curves for the cerebellum and striatum.

2.7. Histology

Deeply anesthetized (Zoletil 50 Vet.) minipigs were euthanised 15 months post MPTP with an intracardial injection of 20 mL sodium pentobarbital 400 mg/mL (Exagon Vet, Richter Pharma, Austria) followed by transcardial perfusion with 5 L of 4% formaldehyde prior to brain removal as previously described (Bjarkam et al., 2017; Ettrup et al., 2011) and stored in 4% formaldehyde at 4 °C. Brains were then embedded in a sectioning chamber (Quick Slicer 100 mm, HistOtech, Aarhus, Denmark) filled with HistOmer mixed with water. Following polymerization of the HistOmer, 2 cm thick sections were cut and transferred to a 30% sucrose in isotonic buffer solution for 1–2 weeks. Brain slabs were then frozen in isopentane cooled to −40 °C for 1–2 min before being cut into eight series of coronal sections (50 μm) on a CryoStar NX70 (Thermo Scientific) and stored at −20 °C. For Nissl-staining, one series was mounted directly onto 0.5% gelatin-coated slides and stained with 0.1% toluidine blue in citrate buffer (pH 4) at room temperature for 9 min followed by a rinse in distilled water, dehydration in 99% alcohol (2×30 s) and clearing with xylene before being coverslipped with Pertex.

Sections covering the substantia nigra pars compacta (SNc) were stained with anti-TH as in Lillethorup et al. (2018a) using TBS containing 0.25% Triton X-100 (TBS-T, 0.05 M, pH 7.4) as a buffer, rabbit anti-TH (1:1000, ab112, Abcam) as primary antibody, a horseradish peroxidase (HRP) labeled secondary antibody (HRP-labeled anti-rabbit, 1:400, Dako) and 0.05% 3,3′-diaminobenzidine (DAB; Alfa Aesar, Cat. no J60972), TBS and 0.01% H_2O_2 for visualization. Sections were then mounted on large 0.5% gelatin-coated slides, cleared in xylene for 5 min and coverslipped with Pertex.

Five slides per animal of anti-TH and Nissl-stained SNc were selected so they covered the rostral to caudal levels (Nielsen et al., 2009; Orlowski et al., 2019) and ten images (5 per hemisphere) were captured using a Nikon light microscope (Nikon, Eclipse, Ni, 1.4 NA) modified for stereology with a digital camera (Olympus DP70, Denmark). A computer with the newCAST software package Visiopharm (Hørsholm, Denmark) was interfaced to the digital camera superimposing stereological probes on the live images. A magnification of 10x was used. ImageJ software was used to semi-quantify the number of TH+ and Nissl-stained cells. Color photographs were taken using a similar exposure time and white balance (850 × 1301 pixels). To allow measurements, photographed sections were converted into 8-bit images followed by semi-automatic segmentation (threshold) to contrast background and stained tissue. After the initial automatic setting, the threshold was manually corrected to ensure that all TH+ and Nissl-positive stains were covered within SNc (threshold between 130 and 160). After that, an irregular rectangle with a fixed area of 5.5 mm^2 was drawn and placed on the SNc region. The

analyze particles plugin was used to automatically estimate the number of stained cells (cells with 2–100 pixels and circularity between 0.5 and 1 were included in the estimation). The average of the 10 measurements for each stain in each minipig was used.

2.8. Statistics

Statistical tests were performed using Prism9 (GraphPad Software, California, USA). To compare behavioral data a repeated measures one-way ANOVA with Bonferroni correction for multiple comparisons was used to compare MPTP animals at baseline with each post-MPTP timepoint. Due to missing values in the PET data, a mixed-effects analysis with Dunnett's multiple comparisons test was used to compare the repeated BP_{ND} and Ki measures at baseline to the 1, 3, 10 and 14-month timepoints post-MPTP. The statistical significance level was set at $p < 0.05$ and normal distribution of the data was verified. In order to test the hypothesis that the animals recovered significantly from MPTP treatment, a mixed-effects analysis with Dunnett's test was performed on the PET data acquired 1 month vs 3, 10 and 14 months after the last MPTP treatment. Pearson correlation was performed to determine the relationship between FDOPA uptake and DTBZ binding at 1, 3, 10 and 14 months after MPTP and between the measures of each of the two PET tracers at 14 months and the TH+ cells.

3. Results

3.1. Mild behavioral changes in response to acute MPTP

During the first weeks after MPTP injections, all seven pigs had mildly reduced mobility, three had abnormal standing/leg positions, one displayed freezing of gait, one had head tremor, and three had abnormal swallowing. All of these symptoms, however, were mild and the total score for each animal ranged from 0 to 2, with only two animals with a score of 3 out of 14 in the first weeks after the MPTP treatment (Fig. 2a). The animals in this study were, therefore, classified as asymptomatic or mildly symptomatic during the most affected period, and did not need treatment of symptoms. No score above 0 was observed in the two saline-injected animals at any time during the study.

Using the GAIT4Dog walking mat, we measured gait symmetry of the minipigs by assessing the Total Pressure Index (TPI) for all limbs, which is expected to be 30% for each front limb (LF and RF) and 20% for each hind limb (LH and RH) under healthy conditions. Both saline (n = 2) and MPTP injected minipigs (n = 7) had TPIs close to the expected 30% for front limbs (range: 28.0–32.5%) and 20% for hind limbs (range: 17.5–20.9%) at all measured timepoints accounting for small individual variances (Table 2).

We also used the GAIT4Dog walking mat to study gait velocity and found a decrease (12%, p = 0.23) in the 7 pigs injected with MPTP one week after the final MPTP injection compared to baseline. By the next testing at 4 months, the gait velocity in all pigs had increased from baseline (10%, p = 0.63), and by 15 months post-MPTP, the values had significantly increased by 29% from baseline values ($p < 0.016$) (Fig. 2b).

3.2. Longitudinal effects of MPTP on neurotransmission

Average parametric Ki and BP_{ND} maps at baseline and 1, 3, 10 and 14 months after MPTP are shown in Fig. 3a and b. MPTP reduced striatal [^{18}F]-FDOPA Ki values at each of the 4 timepoints by an average of 65.7%, 63.7%, 52.4% and 50.4%, respectively, after MPTP injections compared to baseline, which reached significance at 1, 10 and 14 months post-MPTP compared to baseline (Mixed-effects analysis, Dunnett's correction, $p < 0.05$), while no changes were observed in response to saline injections (Fig. 4a). Similarly, MPTP significantly reduced striatal [^{11}C]-DTBZ BP_{ND} values at each of the four timepoints by 58.9%, 59.0%, 54.9% and 55.4%, respectively, after the MPTP injections compared to baseline (Mixed-effects analysis, Dunnett's correction, $p < 0.01$) while saline-injected animals showed no changes from baseline (Fig. 4b).

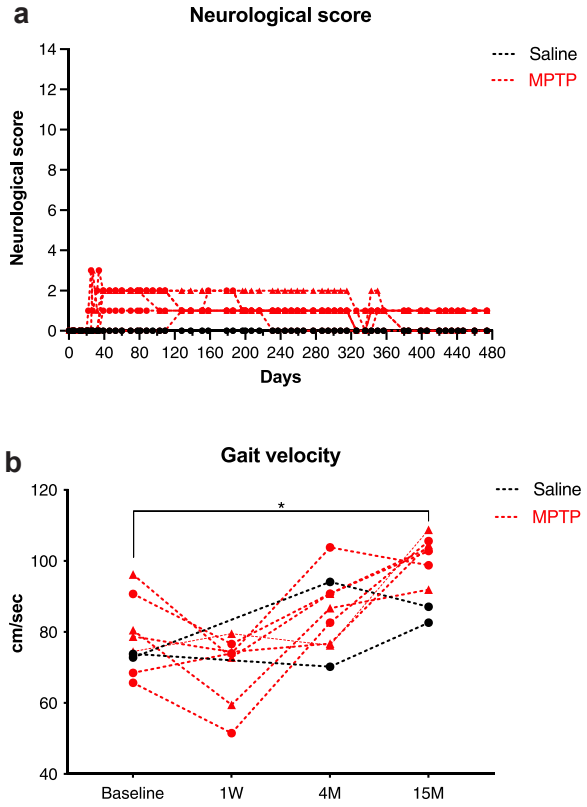


Fig. 2.. a) Neurological score of the minipigs during the 478 day observation period based on motility, coordination, rigidity, chewing and vocalisations, and b)Velocity on the gait mat after MPTP where each data point represents an average of minimum 3 quality walks on the GAIT4Dog walking mat for each minipig at baseline, 1 week, 4 months and 15 months. For both figures, black circles show minipigs receiving saline injections (n = 2) and red circles and triangles represent minipigs injected with MPTP. (n = 7). Note that the MPTP minipigs received either injection of stem cells which did not survive (red circles) or sham surgery (red triangles) in a previous study. Statistical analysis comparing the MPTP animals at baseline vs post-MPTP timepoints revealed a significant increased velocity at 15 months post-MPTP compared to baseline (Repeated measures one-way ANOVA with Bonferroni correction, *$p < 0.05$).

In order to test the impression that the binding values recovered between the time of initial intoxication and the end of the study, we performed a mixed-effects analysis with Dunnett's multiple comparisons test on the data from the 7 minipigs injected with MPTP and compared initial intoxication at 1 months with the 3, 10 and 14 month-timepoints. We found that striatal [^{18}F]-FDOPA Ki values recovered significantly from 1 to 10 (38.6%, $p < 0.05$) and 14 months (44.4%, $p < 0.05$) (Fig. 4a) but with no significant recovery in striatal [^{11}C]-DTBZ BP_{ND} (Fig. 4b).

3.3. Histological confirmation of nigral dopamine cell loss

We estimated the number of TH+ dopamine neurons and the total number of neurons (Nissl-stained cells) in both hemispheres on five corresponding sections covering the SNc per animal. The number of TH+ (Fig. 5a) and Nissl-stained cells (Fig. 5b) per 5.5 mm^2 are shown for each animal (dots) and each group (bar). The number of TH+ cells was reduced by 57% (100.5 ± 33.75) in the MPTP-injected minipigs (n = 7) compared to the saline-injected minipigs (231.1 ± 29.61, n = 2). There was also a decrease of 47% in the number of Nissl-stained cells in the MPTP-injected minipigs (171.5 ± 33.99) when compared to

Table 2
Total Pressure Index (TPI, %) for saline and MPTP-injected minipigs at baseline, 1 week (MPTP only), 4 months and 15 months after the last injection. LF = Left front limb, RF = Right front limb, LH = Left hind limb, RH = Right hind limb.

	Saline (n = 2)			MPTP (n = 7)			
	Baseline	4 mo	15 mo	Baseline	1 week	4 mo	15 mo
TPI% LF	32.3 ± 0.4	31.5 ± 1.6	31.8 ± 1.3	30.6 ± 1.6	29.9 ± 1.0	29.2 ± 1.6	28.0 ± 1.0
TPI% RF	32.5 ± 0.5	32.3 ± 2.6	31.6 ± 1.1	30.3 ± 1.2	30.1 ± 1.2	30.9 ± 1.1	30.2 ± 1.9
TPI% LH	17.9 ± 1.3	18.3 ± 2.1	19.0 ± 2.5	19.7 ± 1.6	20.0 ± 1.2	20.4 ± 1.1	20.9 ± 1.5
TPI% RH	17.5 ± 0.3	17.8 ± 1.8	17.5 ± 0.1	19.4 ± 0.4	20.0 ± 0.7	19.5 ± 0.7	20.8 ± 1.3

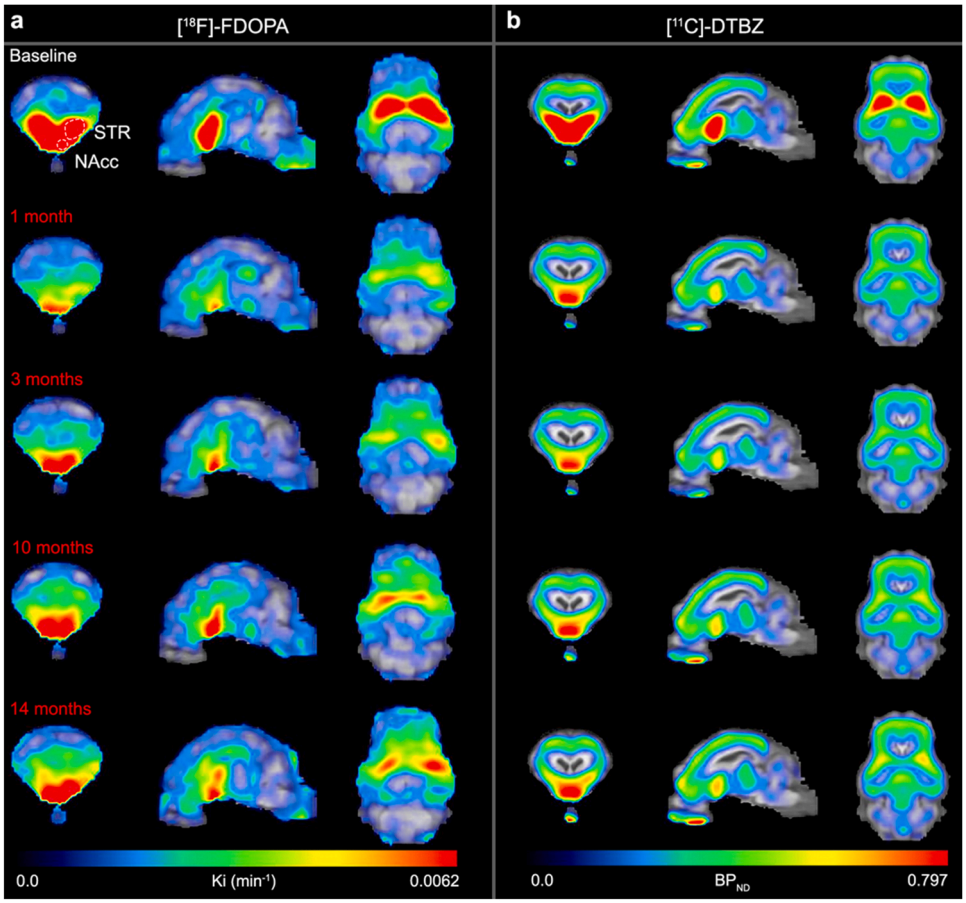


Fig. 3. Average parametric maps of [^{18}F]-FDOPA Ki (a) and [^{11}C]-DTBZ BP_{ND} (b) for minipigs at baseline (top row), 1 month (second row), 3 months (third row), 10 months (fourth row) and 14 months (fifth row) after MPTP injections (n = 5–7). Coronal (left column), sagittal (middle column) and axial/transverse (right column) views are shown for both Ki (min^{-1}) and BP_{ND} maps. NAcc = Nucleus accumbens, STR = striatum, incl. caudate and putamen.

saline-injected minipigs (302.7 ± 41.45), confirming the presence of cell death in the model.

3.4. Correlations

We interrogated whether [^{18}F]-FDOPA uptake and [^{11}C]-DTBZ binding were correlated using measures at each time-point after MPTP (Fig. 6). We found a significant, positive, linear correlation between the binding of the two radioligands at 10 months after MPTP (r^2 =0.834, n = 6, $p < 0.05$) but not at any other timepoint (1 month: r^2 =0.486, n = 7, $p > 0.05$; 3 months: r^2 =0.259, n = 6, $p > 0.05$; 14 months: r^2 =0.604, n = 6, $p > 0.05$).

We then tested potential correlations between binding of each PET ligand at 14 months after MPTP and our estimation of nigral TH+ cells (Fig. 7) and found a significant, positive, linear correlation for [^{11}C]-DTBZ (r^2 =0.844, n = 6, $p < 0.01$) with TH+ cells, but not for [^{18}F]-FDOPA (r^2 =0.321, n = 6, $p > 0.05$).

4. Discussion

We followed longitudinally the behavior and dopaminergic function of Göttingen minipigs exposed repetitively to MPTP with multi-tracer PET imaging. Our data show that minipigs dosed with MPTP over a one-month period have significant reductions in dopaminergic presynaptic function, observed up to 14 months later, along with cell death, even in the absence of sustained behavioral symptoms. Decreased velocity and subtle motor symptoms were only present 1 week after the last MPTP-injection after which time the symptoms stabilized, suggesting that the minipigs were only partially lesioned and that the residual amount of dopamine maintained normal gait. Despite the significant reductions in tracer binding at all timepoints post-MPTP, we found spontaneous partial recovery of striatal [^{18}F]-FDOPA Ki uptake at 10 and 14 months compared to 1 month after the final MPTP injection.

The MPTP lesion model in non-human primates has been widely used, especially for trialing the efficacy of novel symptomatic

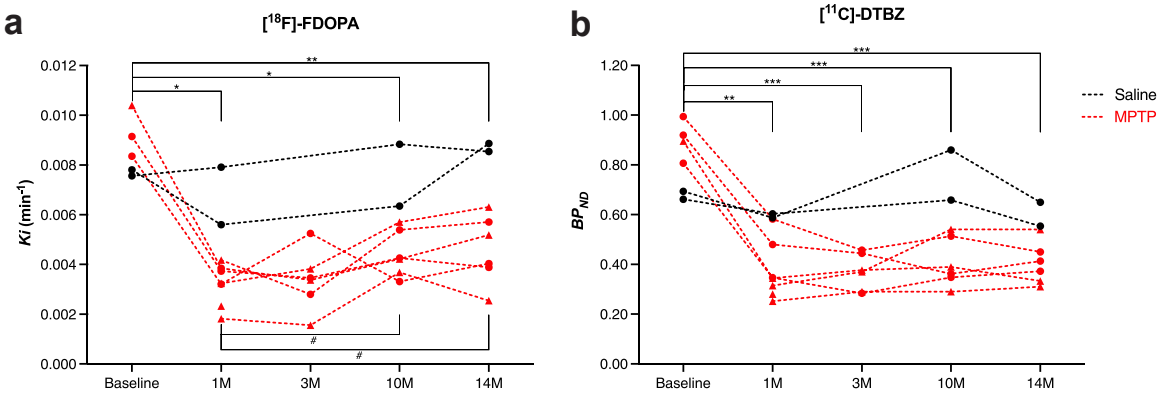


Fig. 4. Longitudinal effects of MPTP on [^{18}F]-FDOPA Ki and [^{11}C]-DTBZ BP_{ND}. Black circles represent the a) striatal Ki (min^{-1}) and b) striatal BP_{ND} values for each animal injected with saline and scanned at baseline and after 1, 10 and 14 months (n = 2), and the red circles and triangles represent the striatal values at 1, 3, 10 and 14 months after MPTP + stem cells or MPTP + sham, respectively (n = 5–7). Statistical analysis comparing the MPTP animals at baseline vs post-MPTP timepoints revealed a significant decreased binding of a) [^{18}F]-FDOPA Ki at 1, 10, and 14 months post-MPTP compared to baseline (Mixed-effects analysis with Dunnett's test, *$p < 0.05$ and **$p < 0.01$) and of b) [^{11}C]-DTBZ BP_{ND} for each time-point post-MPTP compared to baseline (Mixed-effects analysis with Dunnett's test, **$p < 0.01$, *** $p < 0.001$). Striatal [^{11}C]-DTBZ BP_{ND} showed no differences between 1 and 3, 10 or 14 months after MPTP, while [^{18}F]-FDOPA Ki had significantly increased after 10 and 14 months post-MPTP compared to 1 month (Mixed-effects analysis with Dunnett's test, #$p < 0.05$).

medications (Konnova and Swanberg, 2018). However, there are well known limitations to MPTP administration, such as variable dose response, poorly understood compensatory mechanisms and, in particular, spontaneous recovery from motor symptoms (Eidelberg et al., 1986; Taylor et al., 1997), not observed in idiopathic PD patients. This limits its potential to investigate long-term efficacy of putative neuroprotective medications on motor symptoms. Studies have found that cell loss, however, is not statistically different between non-human primates that recover from MPTP exposure compared to those with stable motor deficits (Mounayar et al., 2007) suggesting variable degrees of dopamine terminal adaptation to the nigral lesions are occurring. Indeed, it has been found that remaining TH+ fibers can sprout and increase branching after partial lesion with MPTP in non-human primates (Song and Haber, 2000) and our data suggest an increase in aromatic L-amino acid decarboxylase (AADC) activity or sprouting may be important for the intracellular retention of [^{18}F]-FDOPA in remaining terminals at both 10 and 14 months post-MPTP. Additional studies are necessary to reveal the underlying mechanism of restoration of [^{18}F]-FDOPA. However, other groups have studied the recovery from motor symptoms induced by MPTP in monkeys, and despite considerable striatal dopamine depletion, they report increased striatal dopamine release from remaining fibers and turnover (Petzinger et al., 2006; Schneider, 1990) as well as increased serotonin levels in striatum, also suggesting a role for serotonin neurotransmission in motor recovery (Boulet et al., 2008).

Previous studies in non-human primates administered cumulative doses of 1.6 mg/kg (Ballanger et al., 2016) and 1.8 mg/kg as a progressive intoxication dose, and 3.2 mg/kg as an acute intoxication dose (Mounayar et al., 2007). Our initial dose of 1 mg/kg three times a week, was doubled in the final 2 weeks of injections due to the apparent lack of motor symptoms, resulting in a cumulative dose of 18 mg/kg over 33 days. Other studies of Göttingen minipigs administered MPTP have shown behavioral sensitivity and moderate parkinsonism with a 0.7–1 mg/kg SC injection (cumulative dose of 6–13 mg/kg over 6–10 days (Mikkelsen et al., 1999), 6.6 ± 1.4 mg/kg over a 22-day injection period (Danielsen et al., 2000) and a cumulative dose of 8 mg/kg over 16 days (Dall et al., 2002)). The parkinsonian symptoms demonstrated were in the form of hypokinesia, reduced coordination and muscle rigidity already during the first five days with a behavioral score of 4–8 out of 8 (Mikkelsen et al., 1999), rigid hindlimbs, reduced spontaneous activity and coordination during the first month with behavioral score of 3–8 out of 12 (Dall et al., 2002) or hypokinesia, muscle rigidity and impaired coordination during the first 7–10 days with a mean behavioral score of 6 out of 12 (Danielsen et al., 2000). A drop in cell numbers in the SN was seen three months after drug administration along with reductions in brain levels of dopamine, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) (Mikkelsen et al., 1999). Still, behavioral recovery was reported during the 90 days after the last MPTP-dose in Mikkelsen et al. (1999) and towards the end of the 6-month study period in Dall et al. (2002).

In human patients, an estimated 50–70% putaminal signal loss by [^{18}F]-FDOPA is seen at PD diagnosis and our results show ~60% loss (Lee et al., 2000; Morrish et al., 1998). This is consistent with changes reported in MPTP-exposed non-human primates (Blesa et al., 2012) and both idiopathic and MPTP-exposed PD patients (Snow et al., 2000). Interestingly, Dall et al. (2002) also report a 60% reduction in the relative activity of DOPA decarboxylase in striatum using [^{18}F]-FDOPA PET following MPTP intoxication in minipigs. It is unknown why our MPTP dose was insufficient to elicit moderate parkinsonian symptoms, or any stable behavioral impairment and instead modeled the prodromal stage of PD. However, another study investigated continuous infusion of MPTP in minipigs for 11 weeks and reported only mild symptoms with remission to normal state in the group of animals that received 6 mg MPTP/day corresponding to a total dose of 462 mg (Nielsen et al., 2016). A recent study in KSP minipigs also reported recovery in MPTP-induced locomotor abnormalities, including decreased velocity, after the last MPTP administration. The recovery was observed 2 weeks after receiving the last dose of 1 mg/kg SC MPTP (1 time/week over 3 weeks, cumulative dose of 3 mg/kg) or 4 weeks after receiving 1 mg/kg SC MPTP (3 times/week over 7 weeks, cumulative dose of 21 mg/kg) (Seo et al., 2020).

These studies are consistent with our lack of significant behavioral symptoms in response to a high cumulative dose of 18 mg/kg in 25-kg minipigs corresponding to a total dose of at least 450 mg. Since our initial MPTP dosing was consistent with the other reported MPTP minipig studies, it is possible that our injection timetable 3-times a week compared to daily or every second day injections reported by the others (Dall et al., 2002; Danielsen et al., 2000; Mikkelsen et al., 1999; Nielsen et al., 2016) may have contributed to the observed differences. Perhaps consistent delivery on consecutive days is important to avoid compensatory behavioral mechanisms in the minipigs.

Furthermore, we speculate that our use of midazolam and (S)-ketamine anesthesia prior to MPTP-injections to increase safety of the staff handling MPTP, which the other reported MPTP minipig studies did not apply, might have protected the minipigs against extensive fiber loss. Midazolam and MPTP are both competitive inhibitors of various cytochrome P450 isozymes, that are involved in the neurotoxicity of MPTP

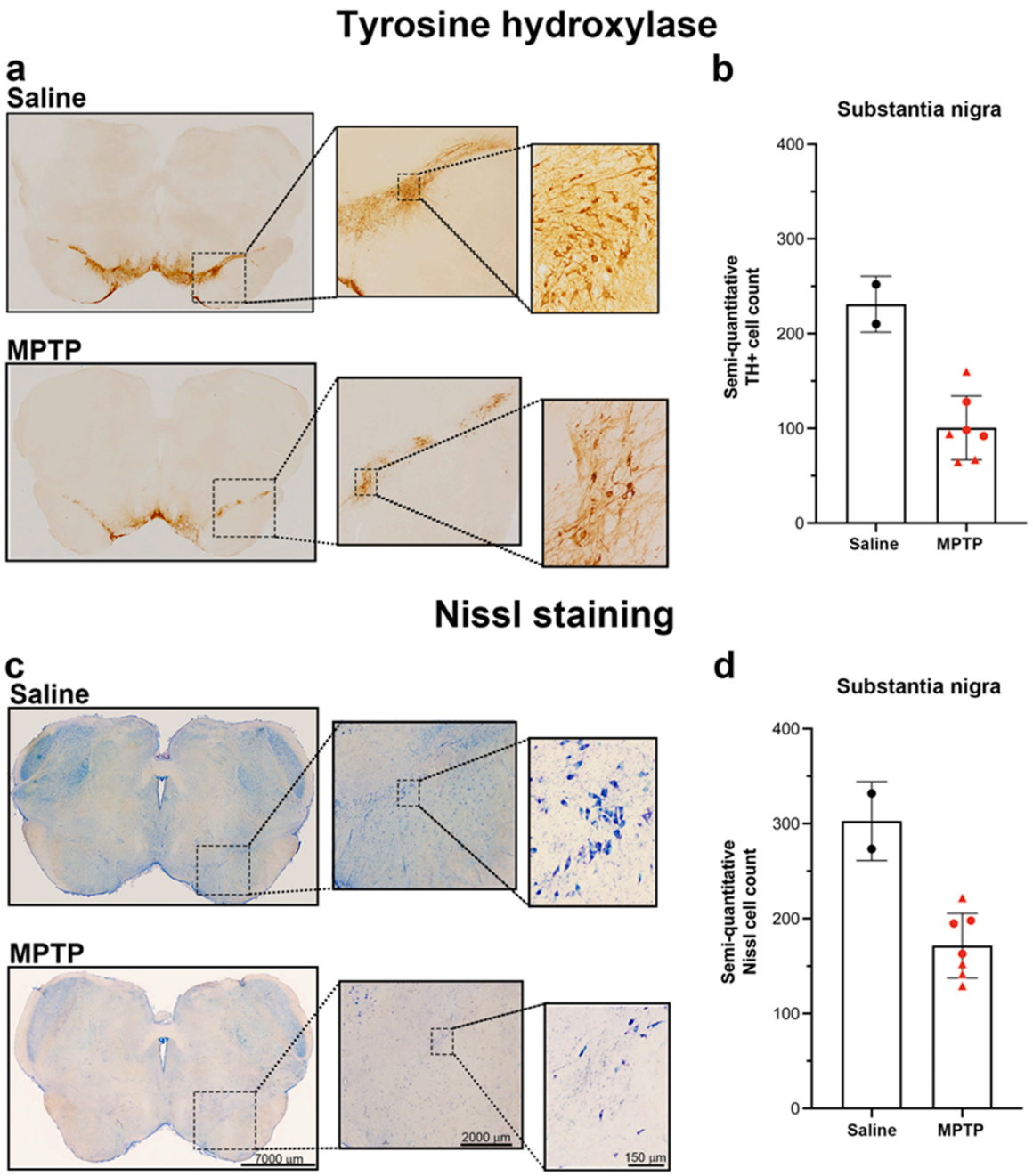
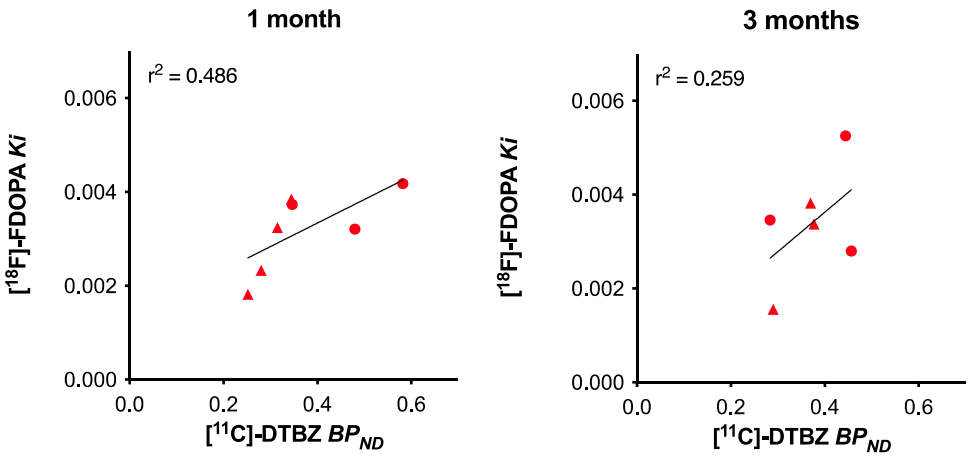


Fig. 5. TH+ cells (a) and Nissl-stained cells (b) were estimated bilaterally in the SNc of the two saline- and seven MPTP-injected minipigs. Each dot in the graph represents the mean of 10 measurements (bilateral data) done in 5 sections using a similar irregular rectangle (5.5 mm^2) covering the SNc for each minipig. Black circles represent saline animals while red circles and triangles represent MPTP + stem cells and MPTP + sham-injected animals, respectively.

(Fonne-Pfister et al., 1987; Wandel et al., 1994). Furthermore, a recent study found that (R)-ketamine attenuated MPTP-induced reduction in striatal dopamine transporter (DAT) and TH, while (S)-ketamine only had a small effect on DAT (Fujita et al., 2020). Therefore, the combination of midazolam and (S)-ketamine may have reduced the dopamine terminal loss. In future work, we would therefore carefully train the minipigs to be restrained in a sling during injections to avoid the possible confounding effects of anesthetics (Zeltner, 2013). Nevertheless, the dose was sufficient to induce an estimated 57% TH+ cell loss postmortem in the SNc and significant reductions in PET markers of striatal presynaptic dopamine neurotransmission. As other studies have shown only phenotype loss to low doses of MPTP (Alam et al., 2017), we estimated the total number of Nissl-stained neurons in the SNc confirming that there was an actual cell loss and not only a reduced TH expression.

[^{18}F]-FDOPA PET has previously been used to assess decline in striatal dopamine terminal function in MPTP-induced parkinsonism and its response to fetal mesencephalic dopaminergic allografts in pigs (Dall et al., 2002) and humans (Ma et al., 2002). The previous transplant study reported motor symptoms and a stable 60% loss of dopa decarboxylase activity 3- and 6-months after MPTP in the non-grafted minipigs, while recovery was reported post-grafting (Dall et al., 2002). We show here that asymptomatic minipigs demonstrate a clear and persistent reduction in [^{18}F]-FDOPA uptake even when behaviorally intact but, for the first time, we also demonstrate a significant recovery at 10 months after MPTP intoxication, which was also sustained at 14 months post-MPTP. The late recovery in [^{18}F]-FDOPA uptake might also explain the increased gait speed observed in the MPTP treated minipigs at 15



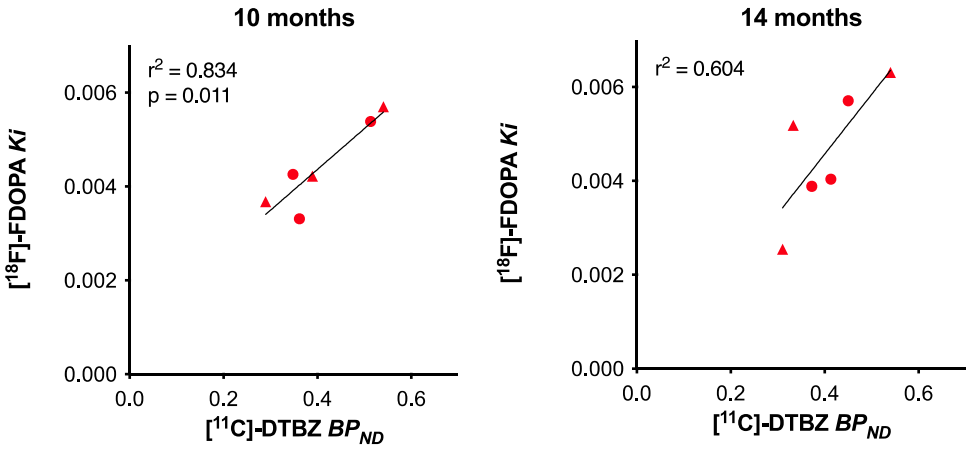


Fig. 6. Correlations between [^{18}F]-FDOPA Ki and [^{11}C]-DTBZ BP_{ND} imaging data at 1, 3, 10 and 14 months after MPTP (n = 6–7). Each data point represents one minipig and red circles and triangles represent MPTP + stem cells and MPTP + sham animals, respectively. The lines are linear fits of the data. [^{18}F]-FDOPA Ki and [^{11}C]-DTBZ BP_{ND} measures exhibited a significant, positive, linear relationship only at 10 months after MPTP (r^2 =0.834, n = 6, p = 0.011). r^2 is shown for each comparison.

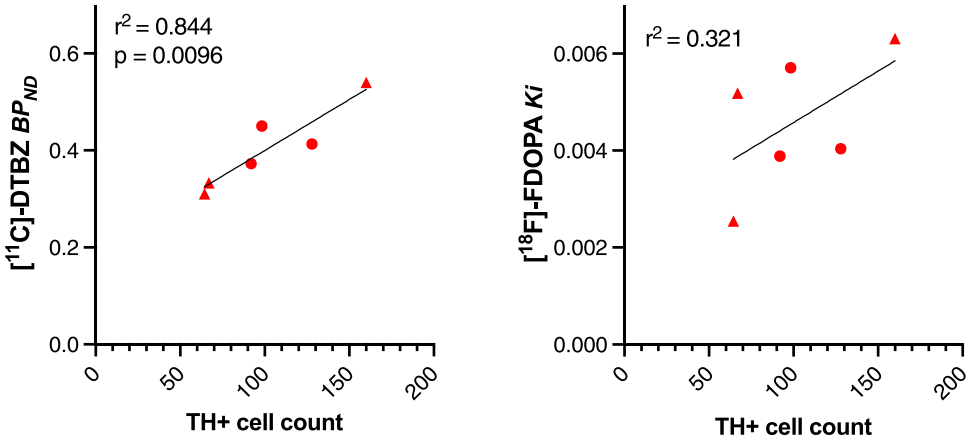


Fig. 7. Correlations between PET imaging data at 14 months after MPTP ([^{11}C]-DTBZ BP_{ND} left panel, [^{18}F]-FDOPA Ki right panel) and mean number of TH+ cells estimated in each hemisphere (area of 5.5 mm^2). Each data point represents one minipig and red circles and triangles represent MPTP + stem cells and MPTP + sham animals, respectively. The lines are linear fits of the data. [^{11}C]-DTBZ BP_{ND} and number of TH+ cells significantly correlated (r^2 =0.844, n = 6, p < 0.01), while [^{18}F]-FDOPA Ki and TH+ cell number did not.

months compared to baseline.

We have previously shown reductions in striatal [^{11}C]-DTBZ binding in minipig models of parkinsonism induced by acute and chronic proteasome inhibition (Lillethorup et al., 2018a; Lillethorup et al., 2018b), and preformed α-synuclein fibrils (Thomsen et al., 2021) or adeno-associated viral (AAV) vector induced α-syn overexpression in rodents (Phan et al., 2017). However, this is the first study of VMAT2 binding in MPTP-injected minipigs. Radio-labeled DTBZ binds with nanomolar-affinity to the VMAT2 transporter protein of synaptic vesicles in monoaminergic neurons(Scherman et al., 1988) and can non-invasively track presynaptic dopamine terminal density in the striatum(Koeppe et al., 1996). It was once thought that DTBZ binding was insensitive to pharmacological challenges (Kilbourn et al., 1996), however, it is now accepted that binding may be altered by changes in intravesicular concentrations of stored dopamine (Kilbourn et al., 2010; Sossi et al., 2010; Tong et al., 2008). Selective loss of VMAT2 is one of the first measurable effects of aging and active PD in human, and aging and MPTP exposure in non-human primates (Chen et al., 2008; Doudet et al., 2006) and precedes the appearance of clinical features. VMAT2 reductions in striatum and SN correlate with disease severity of PD

patients (Hsiao et al., 2014). Here we show 55–60% decreases in VMAT2 availability in MPTP-treated minipigs in the absence of overt motor features of parkinsonism. Unlike [^{18}F]-FDOPA, at the time of the final imaging sessions there was no significant recovery in [^{11}C]-DTBZ binding. Previous studies have found 35–46% loss of VMAT2 in striatal regions of asymptomatic non-human primates 2 months after onset of MPTP exposure which progressed to a 69–78% loss by 10 months (Chen et al., 2008). The different results are likely attributable to the fact that the investigators continued to administer MPTP throughout the entire non-human primate study, whereas the scans conducted in minipig were done at specific times after the final MPTP administration in our sub-chronic protocol.

As [^{18}F]-FDOPA Ki reflects blood-brain barrier transport, AADC activity and vesicular storage, whereas [^{11}C]-DTBZ BP_{ND} only reflects synaptic monoamine vesicle availability, the main differences between the uptake and binding should be reflected by the decarboxylation. Using [^{18}F]-FDOPA, [^{11}C]-DTBZ and [^{11}C]-methylphenidate (labeling DAT) PET, Lee at al. have found that [^{18}F]-FDOPA uptake was reduced to a lesser extent than [^{11}C]-DTBZ and [^{11}C]-methylphenidate in PD basal ganglia (Lee et al., 2000). This difference in uptake and binding was argued to reflect up-regulation of AADC activity in the parkinsonian striatum as a plastic mechanism to increase availability of dopamine to postsynaptic dopamine receptors (Lee et al., 2000). Our functional recovery in [^{18}F]-FDOPA uptake at 10 and 14 months post-MPTP might therefore be explained by AADC activity being more prone to up-regulation following dopamine denervation than VMAT2 due to dynamic regulation and compensatory mechanisms (Hadjiconstantinou et al., 1993; Hefti et al., 1985). Furthermore, the significant correlation of striatal [^{18}F]-FDOPA uptake and [^{11}C]-DTBZ binding only at the 10-month timepoint supports the differential behavior of the two PET tracers across time after MPTP-lesioning in minipigs. We show the temporal stability of [^{11}C]-DTBZ binding at 14 months as a marker of terminal damage that correlates with TH+ cell estimation, unlike [^{18}F]-FDOPA uptake. Previous studies of animal models of PD have reported a significant correlation between [^{11}C]-DTBZ binding and TH (Thomsen et al., 2021) and a poor correlation between [^{18}F]-FDOPA Ki and TH (Yee et al., 2001), supporting the use of [^{11}C]-DTBZ rather than [^{18}F]-FDOPA when planning trials of therapeutic agents in MPTP-lesioned animals.

A limitation of this study is the low number of animals used. The high costs and ethical implications of using a large animal model make it difficult to perform studies with a large number of animals in each group. However, a strength of performing longitudinal in vivo PET imaging is the ability to use the animal's own baseline data as a control. Therefore, we prioritized having a larger cohort in the MPTP treatment group and based the majority of our statistical analyses on the 5–7 baseline vs post-MPTP PET scans at each timepoint. We included 2 saline-injected pigs primarily for the collection of behavioral and post-mortem data for direct comparison with the MPTP-injected pigs.

5. Conclusions

In conclusion, we show here for the first time the temporal evolution of two markers of presynaptic dopamine function in a large animal model, the Göttingen minipig, in response to MPTP. Our asymptomatic to mildly symptomatic animals showed similar radioligand changes observed in behaviorally impaired animal models as well as in human PD. The recovery of [^{18}F]-FDOPA binding 10 and 14 months after the final dose of MPTP suggests that caution should be taken when planning investigations of therapeutic interventions, which may be confounded by spontaneous recovery of the dopaminergic system.

CRediT authorship contribution statement

Conceptualization, **A.M.L., K.S., D.J.B.** and **J.C.S.**; Methodology, **A.K.O.A, K.S., M.B.T., H.Z., D.O., T.W.M., A.N.G., E.H.T.N, A.C.S**; Formal analysis, **A.M.L., T.L.P., O.N., C.C.R, D.O., M.W.**; Writing – original draft, **A.M.L, T.L.P.**; Writing – review & editing, all authors.; Visualization, **A.M.L, T.L.P, O.N., C.C.R, M.W.**; Funding acquisition, **A. M.L., D.J.B. and J.C.S.** All authors have read and agreed to the published version of the manuscript.

Funding

This research was funded by the Lundbeck Foundation, Denmark (R211-2015-3784), Parkinsonforeningen, Denmark and Danish Innovation Fund, Denmark (E! 10838 - DOPALAM). The funding sources had no involvement in the conduct of the research or the preparation of the article.

Institutional review board statement

We conducted experiments with ethical approval and in accordance with guidelines established by the Danish Animal Experiments Inspectorate (license 2016−15−0201−00878) and the European Legislation for the protection of vertebrate animals and in compliance with ARRIVE guidelines.

Informed consent statement

Not applicable.

Declaration of Competing Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Data availability statement

Data are available upon reasonable request to the corresponding author.

Acknowledgements

We would like to thank the staff at the Aarhus University Hospital PET Centre for their technical assistance and the Aarhus University Farm for their invaluable help with the animals. A special thanks to Stine Ledet Methmann for performing the weekly neurological scoring and Fenghua Chen for technical assistance. We thank the CENSE group at the Danish Neuroscience Center including Lise Moberg Fitting and Anne Sofie Møller Andersen.

References

Alam, G., Edler, M., Burchfield, S., Richardson, J.R., 2017. Single low doses of MPTP decrease tyrosine hydroxylase expression in the absence of overt neuron loss. Neurotoxicology 60, 99–106. https://doi.org/10.1016/j.neuro.2017.03.008.

Alstrup, A.K.O., 2010. Anaesthesia and Analgesia in Ellegaard Göttingen Minipigs, first ed. Janneurp; Ellegaard A/S,, Dalmose; Denmark, pp. 1–51.

Alstrup, A.K.O., Jakobsen, S., Wegener, G., Hansen, A.K., Doudet, D.J., Landau, A.M., 2010. Anesthesia in animal imaging: Differing effects of propofol versus isoflurane on dopamine 1 receptor binding in Göttingen minipig brain. NeuroImage 52, S181. https://doi.org/10.1016/j.neuroimage.2010.04.147.

Ballanger, B., Beaudoin-Gobert, M., Neumane, S., Epinat, J., Metereau, E., Duperrier, S., Broussolle, E., Thobois, S., Bonnefoi, F., Tourvielle, C., Lavenne, F., Costes, N., Lebars, D., Zimmer, L., Sgambato-Faure, V., Tremblay, L., 2016. Imaging dopamine and serotonin systems on MPTP monkeys: a longitudinal PET investigation of compensatory mechanisms. J. Neurosci. 36 (5), 1577–1589. https://doi.org/10.1523/JNEUROSCI.2010-15.2016.

Bjarkam, C.R., Orlowski, D., Tvilling, L., Bech, J., Glud, A.N., Sorensen, J.H., 2017. Exposure of the pig CNS for histological analysis: a manual for decapitation, skull opening, and brain removal. J. Vis. Exp. 122, 55511. Retrieved Apr 13, from.

Blesa, J., Pifl, C., Sanchez-Gonzalez, M.A., Juri, C., Garcia-Cabezas, M.A., Adanez, R., Iglesias, E., Collantes, M., Penuelas, I., Sanchez-Hernandez, J.J., Rodriguez-Oroz, M. C., Avendano, C., Hornykiewicz, O., Cavada, C., Obeso, J.A., 2012. The nigrostriatal

system in the presymptomatic and symptomatic stages in the MPTP monkey model: a PET, histological and biochemical study. Neurobiol. Dis. 48 (1), 79–91. https://doi.org/10.1016/j.nbd.2012.05.018.

Boulet, S., Mounayar, S., Poupard, A., Bertrand, A., Jan, C., Pessiglione, M., Hirsch, E.C., Feuerstein, C., Francois, C., Feger, J., Savasta, M., Tremblay, L., 2008. Behavioral recovery in MPTP-treated monkeys: neurochemical mechanisms studied by intrastriatal microdialysis. J. Neurosci. 28 (38), 9575–9584. https://doi.org/10.1523/JNEUROSCI.3465-08.2008.

Burns, R.S., Chiueh, C.C., Markey, S.P., Ebert, M.H., Jacobowitz, D.M., Kopin, I.J., 1983. A primate model of parkinsonism: selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Proc. Natl. Acad. Sci. USA 80 (14), 4546–4550. https://doi.org/10.1073/pnas.80.14.4546.

Chen, M.K., Kuwabara, H., Zhou, Y., Adams, R.J., Brasic, J.R., McGlothan, J.L., Verina, T., Burton, N.C., Alexander, M., Kumar, A., Wong, D.F., Guilarte, T.R., 2008. VMAT2 and dopamine neuron loss in a primate model of Parkinson's disease. J. Neurochem. 105 (1), 78–90. https://doi.org/10.1111/j.1471-4159.2007.05108.x.

Dall, A.M., Danielsen, E.H., Sorensen, J.C., Andersen, F., Moller, A., Zimmer, J., Gjedde, A.H., Cumming, P., Danish Neuronal Xenografting, G., 2002. Quantitative [18F]fluorodopa/PET and histology of fetal mesencephalic dopaminergic grafts to the striatum of MPTP-poisoned minipigs. Cell Transpl. 11 (8), 733–746. ⟨http://www.ncbi.nlm.nih.gov/pubmed/12588105⟩.

Danielsen, E.H., Cumming, P., Andersen, F., Bender, D., Brevig, T., Falborg, L., Gee, A., Gillings, N.M., Hansen, S.B., Hermansen, F., Johansen, J., Johansen, T.E., Dahl-Jorgensen, A., Jorgensen, H.A., Meyer, M., Munk, O., Pedersen, E.B., Poulsen, P.H., Rodell, A.B., Moller, A., 2000. The DaNeX study of embryonic mesencephalic, dopaminergic tissue grafted to a minipig model of Parkinson's disease: preliminary findings of effect of MPTP poisoning on striatal dopaminergic markers. Cell Transpl. 9 (2), 247–259. ⟨http://www.ncbi.nlm.nih.gov/pubmed/10811397⟩.

Doudet, D., Gross, C., Lebrun-Grandie, P., Bioulac, B., 1985. MPTP primate model of Parkinson's disease: a mechanographic and electromyographic study. Brain Res. 335 (1), 194–199. ⟨http://www.ncbi.nlm.nih.gov/pubmed/3873977⟩.

Doudet, D.J., Chan, G.L., Holden, J.E., McGeer, E.G., Aigner, T.A., Wyatt, R.J., Ruth, T.J., 1998. 6-[18F]Fluoro-L-DOPA PET studies of the turnover of dopamine in MPTP-induced parkinsonism in monkeys. Synapse 29 (3), 225–232. https://doi.org/10.1002/(SICI)1098-2396(199807)29:3<225::AID-SYN4>3.0.CO;2-8.

Doudet, D.J., Rosa-Neto, P., Munk, O.L., Ruth, T.J., Jivan, S., Cumming, P., 2006. Effect of age on markers for monoaminergic neurons of normal and MPTP-lesioned rhesus monkeys: a multi-tracer PET study. NeuroImage 30 (1), 26–35. https://doi.org/10.1016/j.neuroimage.2005.09.044.

Eidelberg, E., Brooks, B.A., Morgan, W.W., Walden, J.G., Kokemoor, R.H., 1986. Variability and functional recovery in the N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of parkinsonism in monkeys. Neuroscience 18 (4), 817–822. https://doi.org/10.1016/0306-4522(86)90102-8.

Ettrup, K.S., Glud, A.N., Orlowski, D., Fitting, L.M., Meier, K., Soerensen, J.C., Bjarkam, C.R., Alstrup, A.K., 2011. Basic surgical techniques in the Gottingen minipig: intubation, bladder catheterization, femoral vessel catheterization, and transcardial perfusion. J. Vis. Exp. 52, 2652. https://doi.org/10.3791/2652.

Fonne-Pfister, R., Bargetzi, M.J., Meyer, U.A., 1987. MPTP, the neurotoxin inducing Parkinson's disease, is a potent competitive inhibitor of human and rat cytochrome P450 isozymes (P450bufI, P450db1) catalyzing debrisoquine 4-hydroxylation. Biochem. Biophys. Res. Commun. 148 (3), 1144–1150. https://doi.org/10.1016/s0006-291x(87)80252-8.

Fujita, A., Fujita, Y., Pu, Y., Chang, L., Hashimoto, K., 2020. MPTP-induced dopaminergic neurotoxicity in mouse brain is attenuated after subsequent intranasal administration of (R)-ketamine: a role of TrkB signaling. Psychopharmacology 237 (1), 83–92. https://doi.org/10.1007/s00213-019-05346-5.

Hadjiconstantinou, M., Wemlinger, T.A., Sylvia, C.P., Hubble, J.P., Neff, N.H., 1993. Aromatic L-amino acid decarboxylase activity of mouse striatum is modulated via dopamine receptors. J. Neurochem. 60 (6), 2175–2180. https://doi.org/10.1111/j.1471-4159.1993.tb03503.x.

Harding, J.D., 2017. Nonhuman primates and translational research: progress, opportunities, and challenges. ILAR J. 58 (2), 141–150. https://doi.org/10.1093/ilar/ilx033.

Hefti, F., Enz, A., Melamed, E., 1985. Partial lesions of the nigrostriatal pathway in the rat. Acceleration of transmitter synthesis and release of surviving dopaminergic neurones by drugs. Neuropharmacology 24 (1), 19–23. https://doi.org/10.1016/0028-3908(85)90090-5.

Hsiao, I.T., Weng, Y.H., Hsieh, C.J., Lin, W.Y., Wey, S.P., Kung, M.P., Yen, T.C., Lu, C.S., Lin, K.J., 2014. Correlation of Parkinson disease severity and 18F-DTBZ positron emission tomography. JAMA Neurol. 71 (6), 758–766. https://doi.org/10.1001/jamaneurol.2014.290.

Kilbourn, M.R., Butch, E.R., Desmond, T., Sherman, P., Harris, P.E., Frey, K.A., 2010. In vivo [11C]dihydrotetrabenazine binding in rat striatum: sensitivity to dopamine concentrations. Nucl. Med. Biol. 37 (1), 3–8. https://doi.org/10.1016/j.nucmedbio.2009.08.013.

Kilbourn, M.R., Frey, K.A., Vander Borght, T., Sherman, P.S., 1996. Effects of dopaminergic drug treatments on in vivo radioligand binding to brain vesicular monoamine transporters. Nucl. Med. Biol. 23 (4), 467–471 https://doi.org/0969805196000236 [pii].

Koeppe, R.A., Frey, K.A., Vander Borght, T.M., Karlamangla, A., Jewett, D.M., Lee, L.C., Kilbourn, M.R., Kuhl, D.E., 1996. Kinetic evaluation of [11C]dihydrotetrabenazine by dynamic PET: measurement of vesicular monoamine transporter. J. Cereb. Blood Flow Metab. 16 (6), 1288–1299. https://doi.org/10.1097/00004647-199611000-00025.

Konnova, E.A., & Swanberg, M. , 2018. Animal models of Parkinson's disease. In: Stoker, T. B., Greenland, J. C. e. (Eds.), Parkinson's Disease: Pathogenesis and Clinical Aspects (Internet). ⟨https://doi.org/10.15586/codonpublications.parkinsonsdisease.2018.ch5⟩.

Landau, A.M., Alstrup, A.K., Audrain, H., Jakobsen, S., Simonsen, M., Moller, A., Videbech, P., Wegener, G., Gjedde, A., Doudet, D.J., 2018. Elevated dopamine D1 receptor availability in striatum of Gottingen minipigs after electroconvulsive therapy. J. Cereb. Blood Flow Metab. 38 (5), 881–887. https://doi.org/10.1177/0271678×17705260.

Landau, A.M., Alstrup, A.K.O., Noer, O., Winterdahl, M., Audrain, H., Moller, A., Videbech, P., Wegener, G., Gjedde, A., Doudet, D.J., 2019. Electroconvulsive stimulation differentially affects [(11)C]MDL100,907 binding to cortical and subcortical 5HT2A receptors in porcine brain. J. Psychopharmacol., 269881119836212 https://doi.org/10.1177/0269881119836212.

Landau, A.M., Doudet, D.J., Jakobsen, S., 2012. Amphetamine challenge decreases yohimbine binding to alpha2 adrenoceptors in Landrace pig brain. Psychopharmacology 222 (1), 155–163. https://doi.org/10.1007/s00213-011-2632-6.

Landau, A.M., Luk, K.C., Jones, M.L., Siegrist-Johnstone, R., Young, Y.K., Kouassi, E., Rymar, V.V., Dagher, A., Sadikot, A.F., Desbarats, J., 2005. Defective Fas expression exacerbates neurotoxicity in a model of Parkinson's disease. J. Exp. Med. 202 (5), 575–581. https://doi.org/10.1084/jem.20050163.

Langston, J.W., Forno, L.S., Rebert, C.S., Irwin, I., 1984. Selective nigral toxicity after systemic administration of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyrine (MPTP) in the squirrel monkey. Brain Res. 292 (2), 390–394. ⟨http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=6607092⟩.

Lau, Y.S., Fung, Y.K., 1986. Pharmacological effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on striatal dopamine receptor system. Brain Res. 369 (1–2), 311–315. https://doi.org/10.1016/0006-8993(86)90541-x.

Lau, Y.S., Fung, Y.K., Trobough, K.L., Cashman, J.R., Wilson, J.A., 1991. Depletion of striatal dopamine by the N-oxide of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Neurotoxicology 12 (2), 189–199. ⟨https://www.ncbi.nlm.nih.gov/pubmed/1956580⟩.

Lee, C.S., Samii, A., Sossi, V., Ruth, T.J., Schulzer, M., Holden, J.E., Wudel, J., Pal, P.K., de la Fuente-Fernandez, R., Calne, D.B., Stoessl, A.J., 2000. In vivo positron emission tomographic evidence for compensatory changes in presynaptic dopaminergic nerve terminals in Parkinson's disease. Ann. Neurol. 47 (4), 493–503. ⟨http://www.ncbi.nlm.nih.gov/pubmed/10762161⟩.

Lillethorup, T.P., Glud, A.N., Alstrup, A.K.O., Mikkelsen, T.W., Nielsen, E.H., Zaer, H., Doudet, D.J., Brooks, D.J., Sorensen, J.C.H., Orlowski, D., Landau, A.M., 2018a. Nigrostriatal proteasome inhibition impairs dopamine neurotransmission and motor function in minipigs. Exp. Neurol. 303, 142–152. https://doi.org/10.1016/j.expneurol.2018.02.005.

Lillethorup, T.P., Glud, A.N., Alstrup, A.K.O., Noer, O., Nielsen, E.H.T., Schacht, A.C., Landeck, N., Kirik, D., Orlowski, D., Sorensen, J.C.H., Doudet, D.J., Landau, A.M., 2018b. Longitudinal monoaminergic PET imaging of chronic proteasome inhibition in minipigs. Sci. Rep. 8 (1), 15715. https://doi.org/10.1038/s41598-018-34084-5.

Ma, Y., Feigin, A., Dhawan, V., Fukuda, M., Shi, Q., Greene, P., Breeze, R., Fahn, S., Freed, C., Eidelberg, D., 2002. Dyskinesia after fetal cell transplantation for parkinsonism: a PET study. Ann. Neurol. 52 (5), 628–634. https://doi.org/10.1002/ana.10359.

Mikkelsen, M., Moller, A., Jensen, L.H., Pedersen, A., Harajehi, J.B., Pakkenberg, H., 1999. MPTP-induced Parkinsonism in minipigs: a behavioral, biochemical, and histological study. Neurotoxicol. Teratol. 21 (2), 169–175. ⟨http://www.ncbi.nlm.nih.gov/pubmed/10192277⟩.

Morrish, P.K., Rakshi, J.S., Bailey, D.L., Sawle, G.V., Brooks, D.J., 1998. Measuring the rate of progression and estimating the preclinical period of Parkinson's disease with [18F]dopa PET. J. Neurol. Neurosurg. Psychiatry 64 (3), 314–319. https://doi.org/10.1136/jnnp.64.3.314.

Mounayar, S., Boulet, S., Tande, D., Jan, C., Pessiglione, M., Hirsch, E.C., Feger, J., Savasta, M., Francois, C., Tremblay, L., 2007. A new model to study compensatory mechanisms in MPTP-treated monkeys exhibiting recovery. Brain 130 (Pt 11), 2898–2914. https://doi.org/10.1093/brain/awm208.

Nielsen, M.S., Glud, A.N., Moller, A., Mogensen, P., Bender, D., Sorensen, J.C., Doudet, D., Bjarkam, C.R., 2016. Continuous MPTP intoxication in the Gottingen minipig results in chronic parkinsonian deficits. Acta Neurobiol. Exp. 76 (3), 199–211. https://doi.org/10.21307/ane-2017-020.

Nielsen, M.S., Sorensen, J.C., Bjarkam, C.R., 2009. The substantia nigra pars compacta of the Gottingen minipig: an anatomical and stereological study. Brain Struct. Funct. 213 (4–5), 481–488. https://doi.org/10.1007/s00429-009-0217-5.

Nolbrant, S., Heuer, A., Parmar, M., Kirkeby, A., 2017. Generation of high-purity human ventral midbrain dopaminergic progenitors for in vitro maturation and intracerebral transplantation. Nat. Protoc. 12 (9), 1962–1979. https://doi.org/10.1038/nprot.2017.078.

Orlowski, D., Glud, A.N., Palomero-Gallagher, N., Sorensen, J.C.H., Bjarkam, C.R., 2019. Online histological atlas of the Gottingen minipig brain. Heliyon 5 (3), e01363. https://doi.org/10.1016/j.heliyon.2019.e01363.

Petzinger, G.M., Fisher, B., Hogg, E., Abernathy, A., Arevalo, P., Nixon, K., Jakowec, M. W., 2006. Behavioral motor recovery in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned squirrel monkey (Saimiri sciureus): changes in striatal dopamine and expression of tyrosine hydroxylase and dopamine transporter proteins. J. Neurosci. Res. 83 (2), 332–347. https://doi.org/10.1002/jnr.20730.

Phan, J.A., Stokholm, K., Zareba-Paslawska, J., Jakobsen, S., Vang, K., Gjedde, A., Landau, A.M., Romero-Ramos, M., 2017. Early synaptic dysfunction induced by alpha-synuclein in a rat model of Parkinson's disease. Sci. Rep. 7 (1), 6363. https://doi.org/10.1038/s41598-017-06724-9.

Ramsay, R.R., Salach, J.I., Singer, T.P., 1986. Uptake of the neurotoxin 1-methyl-4-phenylpyridine (MPP+) by mitochondria and its relation to the inhibition of the mitochondrial oxidation of NAD+-linked substrates by MPP. Biochem. Biophys. Res. Commun. 134 (2), 743–748. ⟨https://www.ncbi.nlm.nih.gov/pubmed/2868716⟩.

Scherman, D., Raisman, R., Ploska, A., Agid, Y., 1988. [3H]dihydrotetrabenazine, a new in vitro monoaminergic probe for human brain. J. Neurochem. 50 (4), 1131–1136. ⟨http://www.ncbi.nlm.nih.gov/pubmed/3346671⟩.

Schneider, J.S., 1990. Chronic exposure to low doses of MPTP. II. Neurochemical and pathological consequences in cognitively-impaired, motor asymptomatic monkeys. Brain Res. 534 (1–2), 25–36. https://doi.org/10.1016/0006-8993(90)90108-n.

Seo, J., Yeo, H.G., Park, J., Won, J., Kim, K., Jin, Y.B., Koo, B.S., Lim, K.S., Jeong, K.J., Kang, P., Lee, H.Y., Son, H.C., Baek, S.H., Jeon, C.Y., Song, B.S., Huh, J.W., Lee, D.S., Lee, S.R., Kim, S.U., Lee, Y., 2020. A pilot study on assessment of locomotor behavior using a video tracking system in minipigs. Exp. Anim. 69 (1), 62–69. https://doi.org/10.1538/expanim.19-0065.

Snow, B.J., Vingerhoets, F.J., Langston, J.W., Tetrud, J.W., Sossi, V., Calne, D.B., 2000. Pattern of dopaminergic loss in the striatum of humans with MPTP induced parkinsonism. J. Neurol. Neurosurg. Psychiatry 68 (3), 313–316. https://doi.org/10.1136/jnnp.68.3.313.

Song, D.D., Haber, S.N., 2000. Striatal responses to partial dopaminergic lesion: evidence for compensatory sprouting. J. Neurosci. 20 (13), 5102–5114. ⟨https://www.ncbi.nlm.nih.gov/pubmed/10864967⟩.

Sossi, V., Dinelle, K., Schulzer, M., Mak, E., Doudet, D.J., de la Fuente-Fernandez, R., 2010. Levodopa and pramipexole effects on presynaptic dopamine PET markers and estimated dopamine release. Eur. J. Nucl. Med. Mol. Imaging 37 (12), 2364–2370. https://doi.org/10.1007/s00259-010-1581-3.

Taylor, J.R., Elsworth, J.D., Roth, R.H., Sladek Jr., J.R., Redmond Jr., D.E., 1997. Severe long-term 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism in the vervet monkey (Cercopithecus aethiops sabaeus). Neuroscience 81 (3), 745–755. https://doi.org/10.1016/s0306-4522(97)00214-5.

Thomsen, M.B., Ferreira, S.A., Schacht, A.C., Jacobsen, J., Simonsen, M., Betzer, C., Jensen, P.H., Brooks, D.J., Landau, A.M., Romero-Ramos, M., 2021. PET imaging reveals early and progressive dopaminergic deficits after intra-striatal injection of preformed alpha-synuclein fibrils in rats. Neurobiol. Dis. 149, 105229 https://doi.org/10.1016/j.nbd.2020.105229.

Tong, J., Wilson, A.A., Boileau, I., Houle, S., Kish, S.J., 2008. Dopamine modulating drugs influence striatal (+)-[11C]DTBZ binding in rats: VMAT2 binding is sensitive to changes in vesicular dopamine concentration. Synapse 62 (11), 873–876. https://doi.org/10.1002/syn.20573.

Wandel, C., Bocker, R., Bohrer, H., Browne, A., Rugheimer, E., Martin, E., 1994. Midazolam is metabolized by at least three different cytochrome P450 enzymes. Br. J. Anaesth. 73 (5), 658–661. https://doi.org/10.1093/bja/73.5.658.

Watanabe, H., Andersen, F., Simonsen, C.Z., Evans, S.M., Gjedde, A., Cumming, P., DaNe, X.S.G., 2001. MR-based statistical atlas of the Gottingen minipig brain. NeuroImage 14 (5), 1089–1096. https://doi.org/10.1006/nimg.2001.0910.

Weinreb, O., Youdim, M.B., 2007. A model of MPTP-induced Parkinson's disease in the goldfish. Nat. Protoc. 2 (11), 3016–3021. https://doi.org/10.1038/nprot.2007.393.

Yee, R.E., Irwin, I., Milonas, C., Stout, D.B., Huang, S.C., Shoghi-Jadid, K., Satyamurthy, N., Delanney, L.E., Togasaki, D.M., Farahani, K.F., Delfani, K., Janson, A.M., Phelps, M.E., Langston, J.W., Barrio, J.R., 2001. Novel observations with FDOPA-PET imaging after early nigrostriatal damage. Mov. Disord. 16 (5), 838–848. https://doi.org/10.1002/mds.1168.

Zeltner, A. , 2013. Handling, dosing and training of the Göttingen Minipig. In: Ellegaard Göttingen Minipigs A/S. ⟨https://nanopdf.com/download/handling-dosing-and-training-of-the-gttingen-minipig_pdf⟩.